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# RELATION BETWEEN PUCCINIA GRAMINIS AND PLANTS HIGHLY RESISTANT TO ITS ATTACK

By E. C. STAKMAN,

*Head of the Section of Plant Pathology and Bacteriology, Division of Botany and Plant Pathology, Department of Agriculture, University of Minnesota*

## INTRODUCTION

The intimate relations between host plants and uredine parasites were first carefully investigated by H. Marshall Ward. Ward (10, 12)[1] showed that the relation between the brown-rust of bromes (*Puccinia dispersa* Erikss.) and its host plants might be quite variable. When normal infection took place, as Ward pointed out, a very fine adjustment was made between host and parasite, resulting in a vigorous development of the fungus without immediate serious injury to the host. Indeed, the host seemed sometimes not only not to be injured for a long time but even to be somewhat stimulated. However, in some cases the fungus was found to kill some of the host cells very soon after gaining entrance, and the fungus itself grew but little. A wide range of possibilities was found in varying degrees of infection, establishing the general principle that the success of infection depended largely on the closeness of symbiotic relations set up between the host and the parasite.

Gibson (4) showed that the germ tube of a rust fungus might enter practically any plant, but that after entrance it was unable to produce haustoria and consequently could not live. She found that when varieties of chrysanthemum resistant to *Uredo chrysanthemi* Roze were inoculated with spores of this rust the host cells near the hyphæ were killed, the further growth of the hyphæ being thereby inhibited. Maryat (5) found a similar condition existing between *P. glumarum* Er. and Henn. and host plants partially immune to its attack. The writer (8) has shown that various strains or biologic forms of *P. graminis* Pers. may kill shortly after inoculation comparatively large areas of tissues in host plants that exhibit a considerable degree of resistance to the fungus. Unquestionably the host plant in such cases is often hypersensitive to the fungus, since the fungus kills very early much of

[1] Reference is made by number to "Literature cited," p. 198-199.

the host tissue and fails to develop normally, in sharp contrast to the conditions in normal infection. Where extreme incompatibility exists between host and parasite, there is often no externally visible evidence that the fungus has even gained entrance. In fact, it is hardly accurate to speak of the inoculated plant as the host, since the fungus is unable to attain any considerable degree of development in it (7). The work reported in this paper was with such forms and was undertaken in order to determine whether the phenomenon was one of real resistance or an extreme case of hypersensitiveness. Hypersensitiveness is used here to indicate the abnormally rapid death of the host plant cells when attacked by rust hyphæ. It is used in this sense without any implication as to the exact physiological nature of the phenomenon, referring, therefore, only to the facts substantiated by visual evidence.

## FORMS OF THE FUNGUS INVESTIGATED

The forms used for study were the following: Oats (*Avena sativa*) inoculated with *Puccinia graminis tritici;* oats inoculated with *P. graminis hordei;* rye (*Secale cereale*) inoculated with *P. graminis* from *Dactylis glomerata* after three generations on oats; wheat (*Triticum* spp.) inoculated with *P. graminis* from *Dactylis glomerata* after three generations on oats; wheat inoculated with *P. graminis avenae;* and barley (*Hordeum* spp.) inoculated with *P. graminis* from *Dactylis glomerata* after three generations on oats. The rusts used, except that from *Dactylis glomerata*, had been confined to the particular host from which they were taken for at least 20 "generations"—i. e., 20 successive transfers had been made in the greenhouse. The rust from *Dactylis glomerata* was taken from rusted plants in the field, inoculated on oats, and then transferred to oats two successive times.

The forms selected represent extreme cases of incompatibility, as is shown by the following table:

*Results of inoculations with strains of Puccinia graminis*

| Form of rust used. | Plant inoculated. | Number of plants inoculated. | Number of plants infected. |
|---|---|---|---|
| *Puccinia graminis tritici* | Oats | 115 | 0 |
| *Puccinia graminis hordei* | do | 183 | 3 |
| *Puccinia graminis* from *Dactylis glomerata* | Rye | 114 | 0 |
| Do | Wheat | 86 | 0 |
| Do | Barley | 58 | 0 |
| *Puccinia graminis avenae* | Wheat | 283 | 4 |

In the case of the rust from *Dactylis glomerata*, some of the inoculations were made directly from the grass and some after one to three generations on oats. They are all grouped in the table, since the rust apparently was not changed after having grown for some time on oats.

## EXPERIMENTAL METHODS

The plants used for inoculation were grown in 4-inch pots in the greenhouse. When they were 6 to 8 days old they were inoculated with an ordinary flat inoculating needle, special care being taken not to injure the tissues in any way. After inoculation the pots were placed in pans containing a little water and then covered with bell jars for 24 or 48 hours, or, in a few cases, longer. The inoculated portions were placed in killing fluid at periods ranging from 48 hours to 12 days after inoculation. Flemming's weaker solution, medium chromo-acetic acid, and picro-acetic acid were used at various times. The material was embedded in paraffin in the usual manner and was cut from 6 to 11$\mu$ thick and then stained. For staining, Harper's modification of the triple stain and Gram's stain, with a counter stain of eosin, gave the best results.

## HISTOLOGICAL DETAILS OF HYPHAL INVASION

It is probably superfluous to call attention to the fact that when normal infection, such as occurs, for example, when oats are inoculated with *P. graminis* from *Dactylis glomerata*, takes place, the host cells remain at least apparently normal for a considerable length of time. In the early stages of infection, although the leaf tissues in the infected area may become yellowish in color, it is clearly evident to the naked eye that no extensive and rapid killing of tissues is taking place. When such areas are carefully examined in section, the host cells very frequently appear entirely normal. (Pl. XXVIII, fig. 1.) The hyphæ may grow very vigorously, send haustoria into the cells, and branch profusely without destroying the chloroplasts or in other ways injuring the cell. Even after pustule formation has begun, many of the cells just at the edge of the pustule, where the hyphæ are massed in great numbers, still retain their chloroplasts and are apparently normal in other respects (Pl. XXVIII, fig. 2). It not infrequently happens that cells just under a uredinial sorus, even when the fungus has sent numerous haustoria into them, still retain a number of chloroplasts and seem to have suffered no serious injury. Of course, dead cells are found in a heavily infected region, but in no case does the fungus seem to kill quickly and sharply the cells with which it comes in contact. Normal infection has been described and illustrated quite completely by Ward (9), Evans (2), and the writer (8).

When examination is made, however, of the tissues of a plant that has been inoculated with a rust form which does not grow in it normally, very sharp differences are observable within 48 hours after inoculation. Even in the very early stages it is very evident that normal infection is not taking place and that there is a comparatively violent action and reaction between the plant and the parasite.

The sequence of events in resistant or immune forms is very nearly the same in the different forms studied. The germ tubes form appressoria

over the stomatal slits in a perfectly normal manner, send a protoplasmic process through the slit, and then form the substomatal vesicle. The stimulus to entrance may be negative phototropism, since Fromme (3) has found such a response on the part of uredine germ tubes. Forty-eight hours after inoculation infection threads have frequently already grown into the intercellular spaces and have branched quite profusely. The hyphæ are frequently large and very vigorous in appearance. Haustoria are sometimes sent into the cells of the host; in many cases they are large in size and of normal appearance.

Within a short time after the hyphæ become closely appressed to the host-plant cells, there are usually unmistakable evidences of some deleterious influence upon the host cells. The chloroplasts very often seem to be affected first. They may appear slightly corroded at first and somewhat irregular in outline. They may retain their identity for some time, but more often seem to be clumped together in more or less irregular masses. This appearance may frequently be due to a shrinking of the protoplast from the cell wall, especially when the chloroplasts are quite numerous (Pl. XXVIII, fig. 4 and 5). As the process of disintegration progresses, the outlines of the individual plastids become increasingly fainter until they are scarcely distinguishable. Sometimes, however, the outlines are still visible, although there appears only a more or less uniformly staining mass (Pl. XXVIII, fig. 4 and 5). Eventually the outlines of the plastids become obliterated almost entirely, leaving only a fairly homogeneous, uniformly staining, nongranular mass, with little remaining semblance of structure. In such dead cells, however, the very faint outlines of what were probably chloroplasts may be discernible in some part of the cell (Pl. XXVIII, fig. 6). Sometimes the contents of dead cells appear more or less finely granular, the granules being variously disposed. They may occur in irregular clumps, in beadlike chains, or in various other combinations. More frequently the contents are very nearly homogeneous, with only a few scattering granules.

The action does not always depend upon actual contact. It seems sometimes to precede actual fungous invasion, although in no observed case did it occur very extensively far in advance of actual hyphal invasion. The hyphæ often are very closely appressed to the cell wall, and in such cases the action goes on very rapidly. Quite frequently, however, the chloroplasts on one side of the cell will have been destroyed almost completely, while on the other side, away from the hyphæ, they still appear quite normal. Naturally, of course, all sorts of gradations can be found. The ultimate result is, however, usually the same. The chloroplasts disappear, the nucleus shows definite signs of disintegration, the protoplast collapses, and the cell stands out sharply from the normal ones near it.

Variations occur also in the matter of cells attacked. It seems that sometimes the hyphæ grow over or past cells which apparently escape

injury, and kill those at some distance from the original point of infection. In a few cases it was observed that hyphæ grew from the upper to the lower epidermis, killing a few cells about halfway between the two and killing a number of them near the lower epidermis. Such cases are rather exceptional; the death of the host cells usually follows promptly after the hyphæ reach the cells. Whereas in the case of normal infection pustules with very abundant spore production are being formed within about 7 to 12 days from the time of inoculation, in such cases as those described above a few host cells have been killed and the fungus has reached its limit of development within the same length of time.

The hyphæ do not grow much after the death of the cells. In some cases they were found to be surrounded by dead cells as early as 3½ days after inoculation, and they themselves showed distinct signs of extreme unthriftiness—viz, large vacuoles alternating with coarsely granular areas. Other hyphæ appeared very much as do the older portions beneath an old pustule in cases of normal infection. These hyphæ had grown across the substomatal space and had killed all the mesophyll cells bordering on it, but had not completely killed any deeper lying cells, although some of those just beyond the border cells were somewhat affected. Under such circumstances it is conceivable that the fungus may have died from lack of nourishment, since practically all the food material stored in the spore had probably been used up in the growth of the germ tube along the length of about 10 epidermal cells, in the formation of the substomatal vesicle, and in the growth of the numerous infection threads across the substomatal space. It seems quite possible that the fungus, having exhausted the supply of nutrients stored in the spore, precluded the possibility of its further growth by killing very quickly the first cells with which it came in contact, thus shutting off its only source of food material.

The action is not always as rapid and sharp as in those cases just described. Hyphæ at the end of five days from the time of inoculation have sometimes killed most of the cells in their immediate vicinity, but still remain alive, although they are usually not vigorous. Only the tips of the branches retain protoplasm, while the remainder of the hyphæ are completely vacuolated, with apparently no film of protoplasm next to the walls. The tips at this time usually are also vacuolated very distinctly and show very definite signs of approaching death. It seems clear, therefore, that whatever the intimate physiological relations between host and parasite, the death of the host cells is the direct result of the presence of the hyphæ, and that for some reason the hyphæ themselves succumb soon after.

The essential fact is that the fungus gains entrance in the same manner in susceptible and resistant forms, but acts differently thereafter. In susceptible forms it grows vigorously without seriously affecting the host cells for some time. In resistant forms, on the other hand, a very

rapid action results in the almost immediate death of the host cells. The degree of susceptibility is indicated to a certain extent by the rapidity of this action. The more resistant a form, the quicker are a few host cells in the immediate neighborhood of the invading hyphæ killed and the sooner does the fungus itself cease activity. The visual evidence is clear, but the exact interpretation of the results is more difficult. Marryat (5) considers that the hyphæ of *P. glumarum* in an uncongenial host starve on account of the death of the host cells. This may be the correct explanation, but there seems to be a very definite antagonism between the immune plant and the parasite, which may possibly require another explanation. The work of Ward (9–12) on *P. dispersa*, of Spinks (6) on *P. glumarum*, and of the writer (8) on *P. graminis* seems to indicate that immunity and resistance, especially when very marked, are quite independent of the nourishment of the plant, and although this does not necessarily establish the case, it would seem to point to a very fundamental antagonism. On the other hand, Comes (1) states that resistance in wheats is due to the acidity of cell sap. It seems clear, however, that plants nearly or quite immune to *P. graminis* exhibit the same phenomena in more extreme form as do partially or highly resistant forms.

## SUMMARY

(1) When plants practically immune to *Puccinia graminis* are inoculated, the fungus gains entrance in a perfectly normal manner.

(2) After entrance the fungus rapidly kills a limited number of the plant cells.

(3) The fungus, after having killed the host cells in its immediate vicinity, seems unable to develop further.

(4) The relations between plant and parasite in partially resistant and almost totally immune plants are different in degree only.

(5) Hypersensitiveness of the host seems to be a common phenomenon not only among plants somewhat resistant to *P. graminis* but also among those almost totally immune to it.

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## PLATE XXVIII

The outlines in this illustration were made on a level table by the aid of the camera lucida, using the Leitz achromatic objective No. 6 (4 mm.) and Huygheniau eyepiece Leitz No. IV (×10). The detail was studied under a magnification of 1,000.

Fig. 1.—Oats inoculated with *Puccinia graminis* from *Dactylis glomerata* after three generations on oats. Early infection stage, showing haustoria in the two epidermal cells to the right of the stoma and one in mesophyll cell. Host cells normal; infection normal and successful.

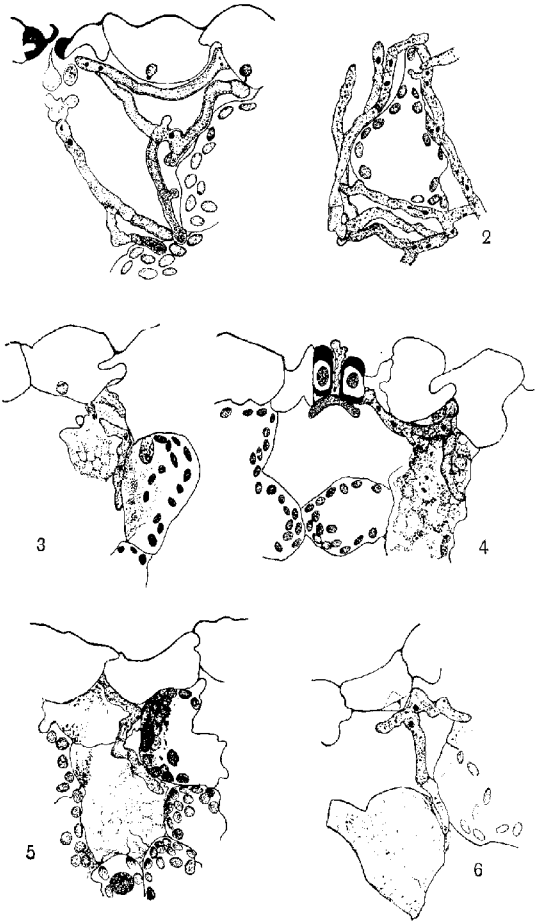
Fig. 2.—Same as figure 1, seven days after inoculation. Cell from edge of pustule area surrounded by hyphæ, but still normal.

Fig. 3.—Oats inoculated with *P. graminis hordei*, four days after inoculation. Piece of mycelium growing over cell; chloroplasts being destroyed; cell at right just being affected; haustorium in epidermal cell.

Fig. 4.—Same as figure 3. Infection thread growing over cell and destroying chloroplasts; normal cells on left.

Fig. 5.—Oats inoculated with *P. graminis tritici* four days after inoculation. Hypha growing over two cells, both of which have been killed; outlines of chloroplasts still showing faintly in second cell; cell to right of hypha becoming affected.

Fig. 6.—Oats inoculated with *P. graminis tritici*, 48 hours after inoculation. The cell on the left killed; outlines of chloroplasts still showing very faintly; cell on the right just becoming affected; tip of hypha dying.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# ANTAGONISM BETWEEN ANIONS AS AFFECTING BARLEY YIELDS ON A CLAY-ADOBE SOIL

By CHARLES B. LIPMAN, *Soil Chemist*, and W. F. GERICKE, *Assistant Soil Chemist*, *Agricultural Experiment Station of the University of California*

## INTRODUCTION

In another publication (4)[1] the senior writer called attention to the results obtained on the antagonism between anions as affecting both the higher plants grown in soils and the bacterial flora in the soils. That brief statement constitutes, so far as we are aware, the first published observation on the existence of antagonism between anions in the case of plants grown in soils. Since the appearance in print of the statement just referred to, there has appeared the work of Miyake (10), which in reporting an elaborate series of experiments confirms the fact first enunciated by us as shown in the discussion below given. Our detailed results were withheld from publication pending such time as complete confirmation of them in repeated experiments in the same soil should make their appearance in print justifiable. These confirmatory data have now been completed, and we submit them below with such discussion as is deemed pertinent and necessary.

As may be inferred from the foregoing statements, there is no literature bearing directly on the subject, with the one exception of the paper noted. As is well known, however, a literature on the general principle of antagonism between ions is rapidly growing voluminous. To this the reader may gain access through Robertson's (14) excellent review of such investigations up to a recent date and through references given by the senior author in recent publications (3, 5) dealing with certain phases of the subject as applied to some of the soil bacteria. In a word, so much evidence has been adduced by various investigators in support of the existence of antagonism between ions for living organisms that we may now more properly speak of it as an established fact of great scientific interest and of practical import rather than as a theory, as heretofore.

In connection with the narrower subject of antagonism between anions, however, we reiterate that very little or nothing has been accomplished. One of the reasons for this is probably to be found in the repeated assertion of the ablest writers on the general subject in question to the effect that anions are of little, if any, importance in a consideration of antagonism between ions. Whatever be the cause, however, there are

[1] Reference is made by number to "Literature cited," p. 217.

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but three reported investigations in all branches of biology, so far as we are aware, which testify to the existence of antagonism between anions, as pointed out by the senior author in one of the papers above cited (3). Two of these were carried out with animals by Moore (11) and Neilson (12) and date back several years. The third is that by Miyake (10) above referred to, which appeared after nearly all of our work was completed and after the statement made by the senior author (4). Our experiments and results therefore constitute a pioneer effort in a virgin field. It is perhaps needless to add that the practical significance of these results when viewed from the standpoint of their bearing on the possible utility of alkali soils for crops is of great moment.

## GENERAL DESCRIPTION OF THE EXPERIMENTS

The experiments were carried out in the greenhouse and the plants were grown in 8-inch paraffined pots. The soil employed was a clay-adobe type found on the campus of the University of California. The plant employed for the experiments was a selected strain of barley (*Hordeum* spp.). Quantities of soil equivalent to about 5 kg. on the basis of dry weight were mixed with the necessary salts, as indicated in the tables, and placed in the pots. The salts employed were the commonest of those of alkali lands—namely, sodium chlorid (NaCl), sodium sulphate ($Na_2SO_4$), and sodium carbonate ($Na_2CO_3$). For the purpose of strengthening the value of our results, two crops in succession were grown in the same pots, allowing the soil a rest period of about three months. Eight seeds were planted in each pot and the plants were later thinned to four to the pot. Water was supplied in quantities as nearly as possible approximating the amount necessary to maintain optimum moisture conditions. Duplicate pots were arranged to represent every one of the treatments. It may be frankly remarked here that results obtained in many of the duplicate pots were far from satisfying. We can not, however, see how these differences, which are brought about by inherent individual plant variations, and by slight differences in the physical condition of the soil in the different pots, could have been avoided. We do not consider our data, therefore, of absolute value and realize further that variations in the technique of our experiments might have yielded better results. Despite all that, however, mixtures of salts allowing the interaction of different anions have permitted, even under much higher osmotic pressures, so much better growth than in smaller concentrations of a single anion only that we feel fully justified in claiming our results to be proof of the undoubted existence of antagonism between anions. Indeed, that is the only claim put forward for our data, but as to its validity we can not see any objection. All other explanatory data are given in the following tables, which are discussed separately and more in detail.

TOXICITY OF THE SINGLE SALTS

It should be stated first in connection with the experiments here described that simultaneously with the series of pots containing salt mixtures of various kinds, in addition to the control plants of every series, several series of plants were also grown for the purpose of determining the degree of toxicity of every one of the salts employed. In these experiments the technique was exactly the same as that employed in the antagonism series. All explanatory data with respect to this added experiment which may be necessary to a comprehension of it are given in Table I, which is presented in full below, despite its very unsatisfactory nature as viewed from the standpoints above discussed.

TABLE I.—*Toxicity of sodium chlorid, sodium sulphate, and sodium carbonate for barley*

| Experiment No. | Sodium chlorid. | | | Sodium sulphate. | | | Sodium carbonate. | | |
|---|---|---|---|---|---|---|---|---|---|
| | Salt added. | Total produce. | | Salt added. | Total produce. | | Salt added. | Total produce. | |
| | | First crop. | Second crop. | | First crop. | Second crop. | | First crop. | Second crop. |
| | *Per cent.* | *Gm.* | *Gm.* | *Per cent.* | *Gm.* | *Gm.* | *Per cent.* | *Gm.* | *Gm.* |
| 1 | 0 | 4.30 | 12.70 | 0 | 4.30 | 12.70 | 0 | 4.30 | 12.70 |
| 1 | 0 | 3.60 | 12.85 | 0 | 3.60 | 12.85 | 0 | 3.60 | 12.85 |
| 2 | 0.10 | 6.70 | 5.60 | 0.10 | 8.40 | 9.30 | 0.05 | 7.00 | 13.95 |
| 2 | .10 | 6.70 | 5.20 | .10 | 7.20 | 8.75 | .05 | 9.20 | 11.30 |
| 3 | .15 | 4.30 | 5.90 | .20 | 7.25 | 7.40 | .10 | 8.00 | 9.40 |
| 3 | .15 | 4.35 | 5.15 | .20 | 6.55 | 9.25 | .10 | 6.80 | 10.70 |
| 4 | .20 | 3.50 | 5.35 | .30 | 7.70 | 10.60 | .15 | 8.20 | 7.80 |
| 4 | .20 | ?6.40 | 6.50 | .30 | 7.40 | 9.00 | .15 | 8.40 | 9.75 |
| 5 | .25 | 4.90 | 5.55 | .40 | 4.65 | 7.30 | .20 | 6.60 | 11.20 |
| 5 | .25 | 3.25 | 5.55 | .40 | 4.90 | 6.30 | .20 | 9.40 | 9.35 |
| 6 | .30 | 3.25 | ..... | .50 | 5.50 | 5.98 | .25 | 7.85 | 11.50 |
| 6 | .30 | 3.30 | ..... | .50 | 5.25 | 5.70 | .25 | 9.10 | 10.32 |
| 7 | .35 | 3.65 | ..... | .60 | 2.08 | 6.30 | .30 | 9.15 | 10.50 |
| 7 | .35 | 3.30 | ..... | .60 | 4.10 | 5.60 | .20 | 9.50 | 14.90 |

Some very interesting points may be gleaned by even a cursory examination of Table I. These may be summarized as follows:

(1) Very little, if any, toxicity is manifested by any of the salts in the first crop.

(2) Decided stimulation is evident in the lowest concentrations employed of every one of the salts in the first crop.

(3) No concentration of sodium carbonate employed gave anything but strong stimulation in the first crop.

(4) Stimulation was almost at a standstill in the sodium-sulphate series at and above the 0.4 per cent concentration in the first crop.

(5) Stimulation was at a standstill in the sodium-chlorid series at and above the 0.15 per cent concentration in the first crop.

(6) The total yields in the second crop throughout are much larger than in the first crop, indicating almost certainly an improvement in the soil and climate during the growth of the first crop and the next period between the two crops.

(7) The toxicity of sodium chlorid and sodium sulphate is plainly discernible in the second crop, even at the lowest concentrations employed. Note the difference between this statement and statement 1.

(8) In the case of sodium carbonate it seems probable that a slight toxicity exists also, even at the lowest concentration. Such toxicity is not nearly so marked, however, as in the cases of the two other salts.

(9) It is remarkable to note the high yields obtained in the sodium-carbonate series of the second crop, as well as the uniformly poor agreement between duplicate pots in the series.

(10) In general, it is a striking fact not easily accounted for that once toxicity does manifest itself it does not seem to become notably more acute as the quantity of salt present increases.

The behavior of the cultures at the lowest concentrations in the first crop is probably to be attributed to an improvement in the physical, chemical, and biological condition of the heavy clay-adobe soil through the salt applications. At the same time the control soils had improved during the growth of the first crop and especially during the period intervening between the two crops much more markedly than the soils treated with the smallest quantities of salts. This improvement was doubtless wrought by good crumb formation in the soil through alternate wetting and drying at first and later by thorough drying during the period of rest. After such improvement, therefore, the control pots showed marked superiority over the pots nearest them in the series which during the same period had changed but slightly, except in the case of the sodium-carbonate series. While, therefore, the yields of the control pots had trebled in the second crop, they remained practically the same at the lowest sodium-chlorid and sodium-sulphate concentrations. It remains true, however, that generally the yields of the second crop were superior to those of the first, a fact to be attributed, in addition to the above-mentioned causes, to the seasonal and climatic differences obtaining between the periods of growth of the two crops.

### ANTAGONISM BETWEEN SODIUM CHLORID AND SODIUM SULPHATE

Table II gives the results obtained in two successive crops in the series of experiments on antagonism between sodium chlorid and sodium sulphate. The toxic quantity of sodium chlorid used throughout was 0.2 per cent, and sodium sulphate was added in varying quantities up to 0.5 per cent.

TABLE II.—*Results of experiments on antagonism between sodium chlorid and sodium sulphate*

| Experiment No. | Sodium chlorid. | Sodium sulphate. | First crop. Tops. | Roots. | Total. | Total average. | Second crop. Tops. | Roots. | Total. | Total average. |
|---|---|---|---|---|---|---|---|---|---|---|
| | *Per cent.* | *Per cent.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* |
| 1 | 0 | 0 | 3.00 | 0.60 | 3.60 | 4.10 | 10.20 | 2.50 | 12.70 | 12.77 |
| 1 | 0 | 0 | 3.50 | 1.10 | 4.60 | | 10.50 | 2.35 | 12.85 | |
| 2 | 0.20 | 0 | 4.00 | .65 | 4.65 | 5.32 | 7.20 | .60 | 7.80 | 7.25 |
| 2 | .20 | 0 | 5.40 | .60 | 6.00 | | 5.90 | .80 | 6.70 | |
| 3 | .90 | 0.10 | 4.10 | .15 | 4.25 | 3.77 | 8.30 | 1.00 | 9.30 | 9.45 |
| 3 | .20 | .10 | 3.10 | .20 | 3.30 | | 8.50 | 1.10 | 9.60 | |
| 4 | .20 | .15 | 4.30 | .45 | 4.75 | 4.72 | 7.80 | .95 | 8.75 | 9.37 |
| 4 | .20 | .15 | 4.50 | .20 | 4.70 | | 9.00 | 1.00 | 10.00 | |
| 5 | .20 | .20 | 4.80 | .35 | 5.15 | 5.27 | 10.00 | 1.30 | 11.30 | 10.52 |
| 5 | .20 | .20 | 5.20 | .20 | 5.40 | | 9.00 | .75 | 9.75 | |
| 6 | .20 | .25 | 2.70 | .25 | 2.95 | 2.55 | 9.40 | 1.00 | 10.40 | 9.60 |
| 6 | .20 | .25 | 2.00 | .15 | 2.15 | | 7.80 | 1.00 | 8.80 | |
| 7 | .20 | .30 | 2.80 | .20 | 3.00 | 2.32 | 9.00 | 1.23 | 10.23 | 10.06 |
| 7 | .20 | .30 | 1.60 | .05 | 1.65 | | 8.30 | 1.60 | 9.90 | |
| 8 | .20 | .35 | 1.70 | .15 | 1.85 | 1.80 | 11.20 | .95 | 12.15 | 11.77 |
| 8 | .20 | .35 | 1.60 | .15 | 1.75 | | 10.30 | 1.10 | 11.40 | |
| 9 | .20 | .40 | 2.80 | .20 | 3.00 | 2.27 | 8.30 | .68 | 8.98 | 9.68 |
| 9 | .20 | .40 | 1.40 | .15 | 1.55 | | 9.40 | .98 | 10.38 | |
| 10 | .20 | .45 | 2.00 | .30 | 2.30 | 2.57 | 7.00 | .60 | 7.60 | 8.66 |
| 10 | .20 | .45 | 2.60 | .25 | 2.85 | | 8.80 | .92 | 9.72 | |
| 11 | .20 | .50 | 1.60 | .10 | 1.70 | 1.70 | 7.30 | .84 | 8.14 | 9.17 |
| 11 | .20 | .50 | ..... | ..... | ..... | | 9.20 | 1.00 | 10.20 | |

It is plain that in the first crop there can have been only the slightest antagonism, if any. It is true, however, that the medium of growth does not seem to have become seriously impaired through the addition of sodium sulphate to the constant quantity of sodium chlorid up to and including 0.2 per cent of sodium sulphate. After that, a very definite depression in growth and yield is noticeable as more sodium sulphate is added, indicating increased toxicity at combinations of 0.2 per cent of sodium chlorid and 0.25 per cent of sodium sulphate, and above.

Quite different conditions confront us in those parts of Table II devoted to the results of the second crop. We note here, as in the foregoing toxicity series (Table I), not only a marked depression in yield in experiment 2 resulting from the presence of 0.2 per cent of sodium chlorid but also a marked improvement in the yield when sodium sulphate is added to the common salt. Thus, while we obtain an average yield of 7.25 gm. of dry matter (tops and roots) when 0.2 per cent of sodium chlorid is present as against 12.77 gm. in the control pots, the yield is increased to 9.45 gm. when 0.1 per cent of sodium sulphate is present with 0.2 per cent of sodium chlorid, and is still further augmented to 10.52 gm. by the presence of 0.2 per cent of sodium sulphate with sodium chlorid. To be sure, as was remarked above, much irregularity exists

among the figures given; nevertheless, it can not be denied that throughout the series all the salt mixtures yield far better results than the 0.2 per cent of sodium chlorid alone. Indeed, only in one salt mixture (one of the pots of experiment 10) does the yield fall as low as in the duplicate pots of experiment 2. In one case, No. 8, the total yield of dry matter, while not equal to that of the controls, is very near the latter, despite the fact that the soil contains 0.55 per cent of total salts. Yet, in the case of No. 2, with considerably less than half the amount of salt present, the yield is depressed approximately 40 per cent below that of the control pots. In the face of such data, erratic as they seem in some respects, there can be no denial of the existence of antagonism between anions. This record is the first, except the preliminary note (4) referred to above, establishing the existence of antagonism between anions for the higher plants *when the latter are grown in normal soils.*

## ANTAGONISM BETWEEN SODIUM CHLORID AND SODIUM CARBONATE

In this series there was again employed the constant toxic quantity of 0.2 per cent of sodium chlorid throughout. The varying antagonistic agent of the last series, however (sodium sulphate), was here supplanted by sodium carbonate. Other explanatory data are recorded in Table III.

TABLE III.—*Results of experiments on antagonism between sodium chlorid and sodium carbonate*

| Experiment No. | Sodium chlorid. | Sodium carbonate. | First crop. | | | | Second crop. | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Tops. | Roots. | Total. | Total average. | Tops. | Roots. | Total. | Total average. |
| | *Per cent.* | *Per cent.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* |
| 1 | 0 | 0 | 3.00 | 0.60 | 3.60 | 4.10 | 10.20 | 2.50 | 12.70 | 12.77 |
| 1 | 0 | 0 | 3.50 | 1.10 | 4.60 | | 10.50 | 2.35 | 12.85 | |
| 2 | 0.20 | 0 | 4.00 | .65 | 4.65 | 5.32 | 7.20 | .60 | 7.80 | 7.25 |
| 2 | .20 | 0 | 5.40 | .60 | 6.00 | | 5.90 | .80 | 6.70 | |
| 3 | .20 | 0.05 | 4.80 | .40 | 5.20 | 5.90 | 7.80 | .73 | 8.53 | 8.19 |
| 3 | .20 | .05 | 6.20 | .40 | 6.60 | | 7.30 | .55 | 7.85 | |
| 4 | .20 | .10 | 4.80 | .20 | 5.00 | 5.17 | 11.40 | .80 | 12.20 | 11.25 |
| 4 | .20 | .10 | 5.20 | .35 | 5.35 | | 9.20 | 1.10 | 10.30 | |
| 5 | .20 | .15 | ..... | ..... | ..... | 2.55 | 12.00 | .30 | 12.30 | 12.35 |
| 5 | .20 | .15 | 2.50 | .05 | 2.55 | | 11.50 | .90 | 12.40 | |
| 6 | .20 | .20 | 2.80 | .05 | 2.85 | 3.25 | 13.50 | .65 | 14.15 | 12.93 |
| 6 | .20 | .20 | 3.60 | .05 | 3.65 | | 10.20 | 1.50 | 11.70 | |
| 7 | .20 | .25 | 4.80 | .20 | 5.00 | 3.87 | 9.00 | .35 | 9.35 | 10.23 |
| 7 | .20 | .25 | 2.70 | .05 | 2.75 | | 10.20 | .90 | 11.10 | |
| 8 | .20 | .30 | (a) | (a) | (a) | (a) | 7.20 | .50 | 8.20 | 10.50 |
| 8 | .20 | .30 | (a) | (a) | (a) | | 12.30 | .50 | 12.80 | |

a No growth.

Studying the data of Table III, evidence of only slight antagonism in the first crop is again seen. It should be noted, however, that, slight as it is, it is much more definite than in the case of the foregoing series.

On the other hand, the increased toxicity which follows the points of antagonism is much more sharply marked in the series immediately under discussion, and only 0.15 per cent of sodium carbonate need be added to 0.2 per cent of sodium chlorid to depress the yield to the point to which it takes an addition of 0.25 per cent of sodium sulphate to 0.2 per cent of sodium chlorid to depress it.

In the case of the second crop, data of very similar nature to those of the foregoing series are noted. The addition of even small quantities (0.05 per cent) of sodium carbonate to 0.2 per cent of sodium chlorid is instrumental in bringing about noticeably better growth, whereas markedly higher results are obtained when amounts of sodium carbonate equivalent to 0.1 per cent of the dry weight of the soil are added to 0.2 per cent of sodium chlorid. The maximum yield is obtained in No. 6, in which the total dry matter produced is even greater than that of the control pots. Even the addition of 0.3 per cent of sodium carbonate produces marked antagonism to the sodium chlorid and allows a good yield. The data in this series are therefore even more emphatically in support of the existence of antagonism between anions than those of the foregoing series involving the interaction of sodium chlorid and sodium sulphate. Thus, again, an increase in the total alkali content of the soils from 0.2 to 0.5 per cent by the addition of 0.3 per cent of sodium carbonate to 0.2 per cent of sodium chlorid, so far from rendering the soil a much poorer medium for plant growth, has made it even better than the control soils containing no salt, and nearly twice as good a producer as the same soil containing the same quantity of sodium chlorid but no sodium carbonate.

### ANTAGONISM BETWEEN SODIUM CARBONATE AND SODIUM SULPHATE

The arrangement of this series was similar to that of the preceding series, sodium carbonate, however, being used as the constant toxic salt and sodium sulphate being used in varying quantities for purposes of antagonism. The results follow in Table IV.

This is the only series of those submitted in this paper which gives no proof of antagonism between anions when two salts are mixed. Apparently there seems to be antagonism in this series not only in the case of the second crop, as in the foregoing series, but also in the case of the first crop. In reality, however, this is not so, as can be seen by an examination of the data submitted in Table I. From the latter we see that the assumed toxicity of culture medium 2 of Table IV is greater than that of any other of the toxicity series for sodium carbonate, even where twice as much sodium carbonate is employed. While, therefore, there may be an actual antagonism between sodium carbonate and sodium sulphate, the evidence of it in Table IV is absolutely untrustworthy, and largely for the reason that no quantity of sodium carbonate employed has actually been shown to be definitely toxic to barley in the clay-adobe

soil. This is in striking contrast to the results given in Tables II and III, which are based on toxic properties of given quantities of sodium chlorid that are shown to be definite and constant in Table I.

TABLE IV.—*Results of experiments on antagonism between sodium carbonate and sodium sulphate*

| Experiment No. | Sodium carbonate. | Sodium sulphate. | First crop. | | | | Second crop. | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Tops. | Roots. | Total. | Total average. | Tops. | Roots. | Total. | Total average. |
| | *Per cent.* | *Per cent.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* |
| 1 | 0 | 0 | 3.00 | 0.60 | 3.60 | 4.10 | 10.20 | 2.50 | 12.70 | 12.77 |
| 1 | 0 | 0 | 3.50 | 1.10 | 4.60 | | 10.50 | 2.35 | 12.85 | |
| 2 | 0.15 | 0 | 5.40 | 1.20 | 6.60 | 6.30 | 7.70 | 1.05 | 8.75 | 8.27 |
| 2 | .15 | 0 | 5.00 | 1.00 | 6.00 | | 6.90 | .90 | 7.80 | |
| 3 | .15 | 0.10 | 4.00 | 1.20 | 5.20 | 6.20 | 6.50 | .95 | 7.45 | 8.66 |
| 3 | .15 | .10 | 6.60 | .60 | 7.20 | | 9.00 | .87 | 9.87 | |
| 4 | .15 | .15 | 7.20 | .45 | 7.65 | 7.65 | | | | |
| 4 | .15 | .15 | | | | | | | | |
| 5 | .15 | .20 | 5.50 | 1.20 | 6.70 | 7.30 | 9.30 | 1.20 | 10.50 | 9.83 |
| 5 | .15 | .20 | 7.00 | .90 | 7.90 | | 8.00 | 1.20 | 9.20 | |
| 6 | .15 | .25 | 4.50 | .65 | 5.15 | 4.93 | 9.00 | 1.30 | 10.30 | 9.60 |
| 6 | .15 | .25 | 4.70 | | 4.70 | | 8.00 | .90 | 8.90 | |
| 7 | .15 | .30 | 4.50 | .50 | 5.00 | 5.45 | 9.50 | .80 | 10.30 | 9.97 |
| 7 | .15 | .30 | 5.50 | .40 | 5.90 | | 8.50 | 1.15 | 9.65 | |
| 8 | .15 | .35 | 6.30 | .50 | 6.80 | 6.48 | 9.00 | 1.45 | 10.45 | 10.63 |
| 8 | .15 | .35 | 5.50 | .65 | 6.15 | | 10.20 | .70 | 10.90 | |
| 9 | .15 | .40 | 4.00 | .20 | 4.20 | 4.95 | 9.00 | .95 | 9.95 | 11.07 |
| 9 | .15 | .40 | 5.50 | .20 | 5.70 | | 11.00 | 1.20 | 12.20 | |
| 10 | .15 | .50 | 6.80 | .10 | 6.90 | 5.55 | | | | |
| 10 | .15 | .50 | 4.00 | .30 | 4.30 | | | | | |

ANTAGONISM BETWEEN CALCIUM SULPHATE AND SODIUM SULPHATE

While this paper was intended primarily to deal with results obtained with the interaction of anions of the common alkali salts, the antagonism between which has been above established, other interesting factors relating thereto deserve brief consideration here. Questions naturally arise in connection with such work involving the relative efficiency of different salts in counteracting a given toxic salt. Is it, for example, reasonable, on the basis of Loeb's experiments (7, 8), to suppose that bivalent ions like those of calcium would be more efficacious in the antagonism of salts with a monovalent cation than another salt with a monovalent cation? This is a practical question of great importance, so far as the subject under discussion here is concerned. For example, it is of importance for us to know whether sodium sulphate, which occurs in such large quantities in our alkali soils, at times singly and at times together with other salts, can be prevented from reacting deleteriously to plant growth in those soils by the application of gypsum. The latter salt of calcium is now much used in practice for purposes of counteracting the

bad effects of sodium carbonate ("black" alkali) and also as a stimulant to certain legumes which are grown for forage. As was demonstrated by Hilgard (1, p. 457–458) in proposing the use of gypsum for the last-named purpose, the following reversible reaction takes place, which accounts for the beneficial effect of the gypsum for reasons too well known to need repetition here.

$$Na_2CO_3 + CaSO_4 = Na_2SO_4 + CaCO_3$$

However, Hilgard also observes (1, p. 458), "of course, gypsum is of no benefit whatever on soils containing no 'black' alkali, but only ('white') Glauber's and common salt." The finality of this expression only emphasizes again what has been noted so often before—namely, the danger that lurks in positive statements, at least in the inexact sciences, no matter how certain their correctness may appear at the time. In the light of the more recent information on antagonism between ions, one would not subscribe to the statement just quoted. It was, indeed, because of the rapidly accumulating data on antagonism between salts that we were led to doubt the finality of Hilgard's statement and to learn by direct experiment the facts in the case.

Accordingly an experiment similar to those above described was arranged, except that sodium sulphate was used in constant toxic quantity of 0.4 per cent and calcium sulphate ($CaSO_4$) in varying quantities to determine whether any interaction occurs between these salts which proves of value to plant growth in such soils as those here described. It will be remembered that we are dealing here with the same anion but with different cations, one of the latter having a higher valence than the others. The evidence of antagonism given above was obtained with the same cation but with different anions. Other information respecting the mode of arrangement of the experiment, as well as the results thereof, is given in Table V.

Table V not only shows the incorrectness of Hilgard's view but evidences most emphatically that calcium sulphate is a very efficient substance for the purpose of preventing the toxicity of sodium sulphate. In this series we have antagonism in the first crop as well as in the second, a phenomenon only very dubiously noted in the foregoing antagonism series. That the relatively large additions of calcium sulphate should continue, like some of the smaller additions, to show an effect antagonistic to sodium sulphate is not surprising, inasmuch as gypsum is a relatively insoluble salt and would therefore not be expected to cause an increased toxicity when added to another salt, as would be the case with the more soluble salts above studied. Two facts are shown in Table V which are very difficult to explain. One is the different point as regards the concentration of salts at which the most marked antagonism occurs in the two crops, and the other is the behavior of small amounts of gypsum

as compared with the larger amounts in the first crop. We are unable to explain satisfactorily why 0.1 per cent of calcium sulphate should in the first crop render 0.4 per cent of sodium sulphate much more toxic than it is alone, and in the second crop virtually inhibit its toxicity. This can not be accidental, since the same result is obtained in another set of duplicate pots differing from those just described only in containing 0.15 per cent of calcium sulphate instead of 0.1 per cent. Similar results have been obtained by the senior writer and Mr. P. S. Burgess in other investigations (6), but they remain as difficult as ever to explain. This case is particularly troublesome, since the same concentration of salts in the same pot gives practically no crop the first season and a very good crop the second season. In the first case the salts show increased toxicity when calcium sulphate is added to sodium sulphate, whereas a few months later the maximum of antagonism is noted with the same salt mixture in the same soil and pot.

TABLE V.—*Results of experiments on antagonism between calcium sulphate and sodium sulphate*

| Experiment No. | Sodium sulphate. | Calcium sulphate. | First crop. | | | | Second crop. | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Tops. | Roots. | Total. | Total average. | Tops. | Roots. | Total. | Total average. |
| | *Per cent.* | *Per cent.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* |
| 1 | 0 | 0 | 3.00 | 0.60 | 3.60 | 4.10 | 10.20 | 2.50 | 12.70 | 12.27 |
| 1 | 0 | 0 | 3.50 | 1.10 | 4.60 | | 10.50 | 2.35 | 12.85 | |
| 2 | 0.40 | 0 | 4.30 | .35 | 4.65 | 4.77 | 6.70 | .60 | 7.30 | 6.80 |
| 2 | .40 | 0 | 4.50 | .40 | 4.90 | | 5.80 | .50 | 6.30 | |
| 3 | .40 | 0.10 | 1.80 | .40 | 2.20 | 2.30 | 9.20 | 1.90 | 11.10 | 11.00 |
| 3 | .40 | .10 | 2.10 | .30 | 2.40 | | 9.00 | 1.90 | 10.90 | |
| 4 | .40 | .15 | 1.30 | .30 | 1.60 | 2.23 | 9.60 | 2.45 | 12.05 | 12.88 |
| 4 | .40 | .15 | 2.30 | .55 | 2.85 | | 7.70 | 2.00 | 9.70 | |
| 5 | .40 | .20 | 6.20 | .70 | 6.90 | 6.40 | ..... | ..... | ..... | ..... |
| 5 | .40 | .20 | 5.60 | .30 | 5.90 | | ..... | ..... | ..... | |
| 6 | .40 | .25 | 5.00 | .70 | 5.70 | 6.10 | 7.00 | 1.30 | 8.30 | 8.70 |
| 6 | .40 | .25 | 5.80 | .70 | 6.50 | | 8.00 | 1.10 | 9.10 | |
| 7 | .40 | .30 | 6.90 | .70 | 7.60 | 7.30 | 9.00 | 2.35 | 11.35 | 10.73 |
| 7 | .40 | .30 | 6.00 | 1.00 | 7.00 | | 8.40 | 1.70 | 10.10 | |
| 8 | .40 | .35 | 5.40 | 1.40 | 6.80 | 6.85 | 7.20 | 1.55 | 8.75 | 10.45 |
| 8 | .40 | .35 | 6.00 | .90 | 6.90 | | 11.40 | .75 | 12.15 | |
| 9 | .40 | .40 | 5.50 | .60 | 6.10 | 5.45 | 6.20 | 1.45 | 7.65 | 8.55 |
| 9 | .40 | .40 | 4.00 | .80 | 4.80 | | 8.00 | ..... | 9.45 | |
| 10 | .40 | .45 | 7.60 | 1.00 | 8.60 | 7.50 | 8.60 | 2.25 | 10.85 | 10.85 |
| 10 | .40 | .45 | 5.00 | 1.40 | 6.40 | | ..... | ..... | ..... | |
| 11 | .40 | .50 | 4.70 | .80 | 5.50 | 5.75 | 8.60 | 2.25 | 10.85 | 11.40 |
| 11 | .40 | .50 | 5.30 | .70 | 6.00 | | 9.70 | ..... | 11.95 | |

Whatever the cause of this puzzling fact may be, the results given in Table V leave no room for doubt as to the power of gypsum to antagonize the toxic effects of Glauber salt (sodium sulphate) in a clay-adobe soil, barley being the plant grown. It must also be noted in this connection

that, at least so far as some seasons are concerned, small quantities of gypsum are as efficient for the purpose as larger quantities, if not more so. Indeed, this would seem to reply to one of the questions above raised as to the rôle of valence of ions in antagonism. It appears that small quantities of calcium are as efficacious in antagonism to other ions as large quantities of sodium or other univalent ions—or even more so.

## GENERAL DISCUSSION OF THE EXPERIMENTS

Several questions arise in connection with the discussion of the foregoing experiments which deserve brief attention here.

### GRAIN YIELDS AND ROOT PRODUCTION AS RELATED TO ANTAGONISM

Thinking that it might be possible to correlate grain or straw yields with antagonism between ions or with the lack thereof, we proceeded to determine separately from the total weight of the tops of the barley plants the weight of the grain produced. No regularity in the production of grain with respect to soil treatment was found. At times the treatment which yielded the largest amount of dry matter would also be found to give the highest grain yields, but very frequently no such relation could be established. If anything general could be stated with reference to the grain yields of the barley plants, it would be that grain production was nearly uniform throughout the series in any given season. No significance can be found, therefore, to judge from our figures, in the grain yields as criteria of absence or presence of antagonism.

The case is not similar with respect to root production. No matter how marked antagonism may be, as judged by the production of total dry matter, root development from the absolute standpoint is always markedly depressed in the presence of salts in the soil. To be sure, the root production is often improved when total yields are increased, but never in the same proportion, and it will be noted throughout in the tables that the root production is always largest in the control pots. Moreover, in the case of the root growth as in that of grain yield, we find great irregularity, for much of which we are unable to account.

### COMPARISON OF OUR RESULTS WITH THOSE OF OTHER INVESTIGATORS

As stated above, the only other results, so far as we are aware, which have been obtained in antagonism work with anions are those of Miyake (10), which were published after our preliminary statement had appeared. Even Miyake's work, however, gives no results of the antagonism between anions as noted in soil cultures, for all his experiments of this kind were carried out in solutions. Nevertheless, the general nature of the work of the Japanese investigator may here be mentioned for comparison with ours and in confirmation of the latter. Miyake found that for the rice plant (*Oryza sativa*) grown in culture solutions antagonism is apparent

between the $-Cl$, $-SO_4$, and $-NO_3$ ions; that such antagonism, however, is not as marked as that between cations; and that the antagonism of one ion to another—for example, $-SO_4$ to $-Cl$—may be greater than under opposite conditions—e. g., $-Cl$ to $-SO_4$. Likewise, as between $-NO_3$ and $-SO_4$, the former ion has the superior power to neutralize the toxic effects of the other. In so far as antagonism between the anions is observed by Miyake, he confirms by less striking examples in culture solutions what we have shown takes place in soil cultures. We can not agree, however, on the basis of our results that antagonism between anions is more feeble than that between cations. The differences found by Miyake in the power of two anions to counteract mutually each other's toxicity has been pointed out in relation to the nitrifying bacteria by the senior writer and Burgess in another place (5).

Our great caution in pointing out differences between soil and solution cultures is explained in several different publications, some of which are cited in this paper. It must be remembered, moreover, that on the basis of direct comparison of the soil and solution cultures Kearney and Cameron (2) pointed out several years ago the very material differences obtaining between all phases of salt effects in solutions and in field experiments. The importance of this point in investigations of salt effects on living organisms which are intended ultimately for practical application can not be overemphasized.

## MAINTENANCE OF THE ALKALI CONTENT IN THE EXPERIMENTAL SOIL

In anticipation of queries with reference to the maintenance of the original "alkali" concentration in the soils described throughout the experiment the following statement is made. The irrigation was so carried out that drainage from the soils was never noted. In other words, enough water was supplied to provide the plants with all the moisture necessary, but no excess was employed. By keeping glazed plates beneath the pots it was possible to note constantly the lack of percolation from the pots. Moreover, samples of soil were removed from the pots at the end of the second season of growth and analyzed for "alkali." It was always possible to recover all or very nearly all the sodium chlorid and sodium sulphate that had been originally added. Sometimes the quantity recovered showed slightly less and at other times slightly more than was added. These irregularities are doubtless due to the slightly imperfect mixing of the salts with the soil or are errors inherent in the method of determination employed.

Quite the contrary was true, however, of the pots receiving sodium carbonate. Not only was it impossible to recover all the sodium carbonate that had been added at the commencement of the experiment, but it was actually possible to recover very little of that salt, the highest percentage recovered being about 25 per cent of the amount first added. This would

perhaps in part explain the peculiar behavior of this salt in the toxicity series which we have discussed, as well as its behavior in the antagonism series; for small quantities of sodium carbonate, which evidently are all that can be counted on to remain in the soil any length of time, might well act as stimulants rather than as toxic agents. This view is to be considered in conjunction with those above discussed on the behavior of sodium carbonate. However that may be, the slight recovery of this salt effected by us from the treated pots would seem to support even more strongly the view expressed above, in which an analogy is drawn between the behavior of sodium carbonate and that of magnesium carbonate ($MgCO_3$) as first explained by MacIntire (9).

## PRACTICAL CONSIDERATION OF THE EXPERIMENTS

It appears plain, in view of the results of Miyake (10) and ourselves (3-6), that the establishment of the existence of antagonism between anions is invested with at least a certain measure of practical importance. In the State of California, as well as in several other of our Western States, very large acreages of land are to be found in which the predominance of one salt, frequently Glauber salt or common salt, makes impossible successful cropping. It would appear from the above results that it would not be a difficult matter to establish a mode of treatment which would involve the neutralization of the toxic effects of any one or even two of the alkali salts by another alkali salt. Thus, we frequently find soils containing, besides small quantities of other salts, about 0.5 per cent of sodium sulphate. It is clear that in a heavy soil, at least by additions of gypsum at the rate of about 2 tons to the acre or common salt in smaller quantity, we could change the soil from a very poor into a normally producing one, despite the fact that we have very considerably increased the total salt content thereof.

## THEORETICAL CONSIDERATIONS OF THE EXPERIMENTS

Several questions of interest, at present merely in their theoretical aspects, arise from the foregoing discussion and the results which form the basis thereof.

The differences in yields of two successive seasons in the same soils and pots are probably to be largely, though not entirely, attributed to temperature and atmospheric variations. To judge from the data submitted in the tables, the change in the soil's condition from one season to another has operated only in a minor way toward crop improvement. On the other hand, it is not impossible to regard the results as indicative of the opposite condition if particularly great stress is laid on the yields of the control pots during the second season.

The causes of antagonistic action still remain the topics of investigation most difficult of solution. Our results can only point indirectly to possible solutions of this important question. It is, however, interesting to

note that the nitrifying powers of soils were always found to be far superior in those containing mixtures of salts favorable to barley growth. There appears to be a direct relation, therefore, between the nitrate-nitrogen supply and barley growth, as pointed out by the senior writer elsewhere (4); and, further, in view of our specific tests in connection with the experiments under discussion and others, there seems to be a direct relationship between the qualitative and quantitative salt relationships in a soil and its nitrifying power. Is it not just possible, therefore, that in one important respect at least antagonism between ions in soils is attributable to the improved conditions brought about in the nitrate supply? One important question, however, would still remain: Why does a certain salt combination improve the nitrifying power of a soil? This question may perhaps be solved by methods now being employed by Loeb (7, 8) and Osterhout (13), but the answer thereto still appears to be very remote.

The puzzling fact, which has been referred to above, of the difference in effect of a single salt on barley in the same soil in two successive crops permits some interesting theoretical considerations. It appears possible that the stimulating effect noted in the first crop as proceeding from the addition of the lowest quantity of every one of the salts is to be attributed indirectly to a physical improvement in the heavy clay-adobe soil for reasons too well known to soil scientists to need discussion here; in other words, the yield of dry matter obtained with additions of 0.1 per cent of sodium chlorid and 0.1 per cent of sodium sulphate is to be regarded as representing the algebraic sum of the improvement in the soil's physical condition through the action of the salt and the depression in growth through direct influences on the barley plant and indirectly on the soil bacteria. Assuming, however, that the improvement wrought in the soil's physical condition is a greater factor for crop improvement in this case than the last-named effects are for the depression of plant growth, one would naturally expect that the results of the interaction of the two phenomena must be to produce a larger crop in salt-treated soil than is produced in the untreated control soil. The next question will be, therefore, How can one account for the remarkable improvement in the yield of the control soil in the second crop? This, it appears to us, is explicable on the basis of a gradual improvement in the control soil during the growth of the first crop through root action and appreciable changes in contraction and expansion, resulting in better crumb structure; but more completely through a physical improvement of the thoroughly mixed, dry control soil, which is allowed to bake in the loose condition for three months or more between the two crops. The crop produced in the control pots during the second season therefore has all the advantages of physical soil improvement, or many of them, possessed by the salt-treated soil during the first season, and in addition is free from disadvantages introduced by the salt, as explained above. On the basis of

such conceptions, which we offer as a tentative explanation, it seems easy to see why improvement is at first wrought in the clay-adobe soil by the sodium-chlorid and sodium-sulphate treatment and later why a depression is produced by the same salt treatment in the second crop. Actually the toxic effect appears to be there from the beginning but is obliterated by the good effects on the physical condition of the soil wrought by the salt. Given a good physical condition in the soil, however, the toxic effect of the salt becomes easily manifest.

Another matter of interest arises in connection with the behavior of sodium carbonate. It will be noted in the discussion of the last paragraph that the effects of sodium chlorid and sodium sulphate only are considered and not sodium carbonate. This is done advisedly, since an examination of Table I will show that sodium carbonate acts in a different way from the other salts, especially in the second crop. This, it would appear to us, is to be explained on the basis of the distinctive effects of that salt in a chemical and physiological way. As explained by the senior writer in other publications sodium carbonate is a stimulant to ammonification and a deterrent to nitrification. It is possible, therefore, and this is offered merely as a speculation, that stimulated ammonification may result in the direct absorption by the barley of ammonia instead of nitrate; and, if ammonia can be readily assimilated by the barley plant, the large amount of ammonia produced by the soils treated with sodium carbonate should cause marked vegetative development owing to better nitrogen feeding; hence, more dry matter. Moreover, other important considerations enter into this problem. Sodium carbonate is readily transformed into other forms when it is mixed with the soil and carbon dioxid ($CO_2$) is given off in accordance with the same principle which MacIntire et al. (9) have shown to apply to magnesium carbonate when it is mixed with the soil. We should thus obtain other compounds of sodium, probably silicates of that element, which would react differently from sodium carbonate. The marked solvent effect on soil minerals, moreover, which is possessed by this salt would seem to indicate a larger supply of available plant food in the soils treated with this salt and, hence, better plant growth. All of these beneficial effects of sodium carbonate could far outweigh its detrimental effects on the physical condition of the soil and yield the results noted. Indeed, in our more recent work we have obtained results that render questionable the great powers attributed to this salt in destroying the physical condition of all soils or of affecting plants deleteriously.

Pursuant to the last-mentioned idea, it is not out of place here to state in general that the direct toxic effect on plants of the "alkali" salts under consideration has been much exaggerated. The question of alkali tolerance by plants would appear in the light of our recent experiments to resolve itself really into one of alkali tolerance by soils. It is the effect of salts on the latter that is more likely to result seriously for plants than the

interference of salts directly with the normal functioning of plants. It may further be added that our experiments convince us also that even the effects of salts on soils are of an indirect nature, and, with the exception of cases of soils containing 0.75 per cent of total salts or more, they do not offer very serious practical problems in reclamation. This last remark is offered tentatively only as a hope for the practice of alkali-land reclamation, but at present seems well supported in fact.

The curious behavior of gypsum in the first crop, Table V, may be explained from the theoretical standpoint as a result of the fixation of bases, which in turn would change the nature of the soil solution. For example, a relatively small quantity of gypsum, which relatively is a slightly soluble salt, would set free by exchange of bases a certain amount of magnesium in the soil solution. Magnesium, as has been shown by several investigators, is detrimental in some concentrations to plant growth. This might therefore point to a direct toxic effect of magnesium resulting from an application of calcium sulphate. When, however, much more of the latter salt is added to the soil, an excess of calcium is introduced which neutralizes the toxic effects of the magnesium as well as of the sodium sulphate present, and the growth of barley is very much improved. This is offered merely as a speculation of interest and perhaps of significance in connection with the phenomenon noted in the series given in Table V. We recognize in some ways the inadequacy of the foregoing explanation and are not unaware of the flaws in the theory, but we feel that it may lead finally to an explanation of the facts noted.

In concluding the discussion, we desire to state that many other considerations of a theoretical nature enter into the subject of antagonism between anions in soils. The latter are such complicated media, however, and involve so many changes of an intensely complicated nature, that it would be impossible to discuss all these questions here.

## SUMMARY

Results are given above which establish for the first time, so far as we are aware, the existence of antagonism between anions in a clay-adobe soil for barley as follows:

(1) Antagonism is shown between sodium chlorid and sodium sulphate and between sodium chlorid and sodium carbonate in the second crop. None is shown in the first crop.

(2) Slight antagonism is shown between sodium carbonate and sodium sulphate in the first crop. It is questionable whether any exists at all in the second crop.

In subsidiary experiments the following points are established in addition to those named above.

(1) Marked antagonism exists in both the first and second crop between sodium sulphate and calcium sulphate in soil cultures. This has not been considered possible hitherto by Hilgard.

(2) In testing the toxicity of single alkali salts it is found that 0.1 per cent each of sodium chlorid and sodium sulphate stimulates barley in the first crop and reacts poisonously to it in the second crop.

(3) Sodium carbonate does not manifest toxicity, but, on the contrary, shows stimulation even up to concentrations equal to 0.3 per cent of the dry weight of the soil.

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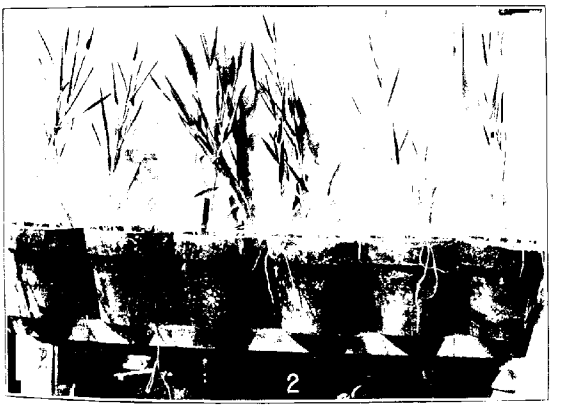
## PLATE XXIX

Barley plants showing growth as affected by various salts in clay-adobe soil.

Fig. 1.—*A*, *B*, Growth with 0.2 per cent of sodium chlorid alone. *C*, *D*, Growth with 0.2 per cent of sodium chlorid and 0.2 per cent of sodium carbonate. *E*, Growth with 0.2 per cent of sodium carbonate alone.

Fig. 2.—*A*, *B*, Growth with 0.2 per cent of sodium chlorid alone. *C*, *D*, Growth with 0.2 per cent of sodium chlorid and 0.5 per cent of sodium sulphate. *E*, *F*, Growth with 0.2 per cent of sodium sulphate alone.


A B C D E




A B C D E F

# A NEW WHEAT THRIPS

By E. O. G. KELLY,

*Entomological Assistant, Cereal- and Forage-Insect Investigations, Bureau of Entomology*

## INTRODUCTION

A new wheat thrips, *Prosopothrips cognatus* Hood, was described by Mr J. D. Hood,[1] of the Biological Survey, from material collected by the writer in 1908. As it is a new species without synonyms, there are, up to this time, no records of its having been destructive. The data contained in this paper have been collected during the last few years whenever the insect occurred in numbers sufficient for study. This species frequently becomes injurious to wheat (*Triticum* spp.) in localized areas, but has not yet been found doing injury to oats (*Avena sativa*) or other grain crops.

## DISTRIBUTION OF THE SPECIES

This wheat thrips occurs in all parts of Kansas, even to the western border; in Oklahoma; at two places in western Missouri; and in one locality in extreme southern Nebraska. Careful search has been made for it in northern Texas, eastern New Mexico, and western Nebraska, and in Kentucky, Tennessee, and Georgia, with negative results.

## DESCRIPTION AND LIFE HISTORY

### THE EGG (PL. XXX, FIG. 1)

The female deposits her eggs in the tissue of the young leaves, usually on the ventral side, whether in wheat or grass, by first cutting the tissue with her sharp mandibles, then thrusting the short ovipositor into the lacerated leaf and placing a single, tiny egg in each puncture.

The egg when first deposited is translucent and nearly colorless, taking on a greenish tinge just before hatching. It is somewhat kidney-shaped, about 0.35 mm. in length, and 0.125 mm. at its greatest diameter.

The hatching period varies from 6 to 10 days and is about the same in the laboratory as under natural conditions in the field.

### THE LARVA (PL. XXX, FIG. 2)

The greenish tinge observed a few hours previous to the issuing of the tiny, slender larva remains with the larva until after it begins to feed, when the color changes to a deeper green as the juices of the plant are imbibed.

[1] Hood, J. D. Prosopothrips cognatus, a new North American Thysanopteron. *In* Canad. Ent., v. 46, no. 2, p. 57-59, fig. 13. 1914.

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When newly hatched, the larva is from 0.39 to 0.40 mm. in length and from 0.09 to 0.10 mm. in width; when full grown it becomes about 1.0 to 1.2 mm. in length. In general shape the body is fusiform, broadest at the middle of the abdomen, and tapering to a point at the anal end. The antennæ are slender, their length being equal to one-sixth that of the body; the bases of the segments are shaded and set in proximal tubercles. There are no changes in the form during growth, except that the color becomes lighter during the last few days of the larval period. The larvæ become full grown in from 10 to 12 days and crawl down the plants into the soil, where they pupate and transform to adults. There is no indication of the yellow color of the adult when the larvæ enter the soil. The pupal period lasts from 10 to 13 days, though no observations on this stage have been noted.

## THE ADULT (PL. XXX, FIG. 3)

The adult, which was described by Hood,[1] may be readily recognized by the fact that it is a wingless form and has a pronounced yellow color, which distinguishes it from *Euthrips tritici* and other species of thrips commonly found on wheat.

## NUMBER OF GENERATIONS ANNUALLY

The complete life cycle from egg to adult requires from 30 to 35 days. The egg hatches in from 6 to 10 days, the larval period occupies from 10 to 12 days, and the pupal period from 10 to 12 days, while the newly issued female requires but from two to three days to prepare for egg laying. Although the length of life of an adult has not been definitely determined, a few have lived eight months in the laboratory.

The adults emerge from winter quarters as soon as the warm days of spring arrive, and the females soon begin depositing their eggs. There are from four to five generations each year. These overlap one another, so that adults and larvæ are present at all times, even in late winter. Larvæ are most numerous at heading time in the spring, also about the time volunteer plants come up in late summer, and again in late fall, when the wheat is getting a good start. They continue to feed until the cold weather causes them to go into hibernation.

## CROPS AFFECTED

Growing wheat is the only cereal that is known to have been damaged by this insect, although certain species of grasses are sometimes injured to a slight degree. Wheat plants furnish its principal food from the time the volunteer plants sprout in August until the wheat crop is harvested the following June. During the interval between wheat harvest and the sprouting of volunteer wheat the thrips feed and reproduce on *Agropyron*

[1] Hood, J. D. Op. cit.

*smithii, Elymus canadense, E. virginicus, Syntherisma sanguinalis, Panicum crus-galli,* and *Hordeum jubatum.* They are found at all seasons of the year on these grasses, but more especially during the interval between harvest and wheat sowing.

## INJURY TO PLANTS

The injury is confined to the leaves of young plants (Pl. XXX, fig. 4), unfolding heads and newly formed grains of wheat, and the young unfolding leaves of some grasses.

The method of feeding is similar to that of other allied species—that is, by first puncturing and lacerating the tissues of the upper epidermis of the leaf, or integument of the grain, then sucking out the juices. Both larvæ and adults feed in this manner, changing their point of attack frequently, and thus in a short time a leaf or grain is literally covered with lacerations.

The leaves when attacked by a dozen or more individuals at one time become badly mutilated in a few hours and, owing to the influence of sunshine and wind, soon acquire a "rusty" appearance. Since the injured leaves nearly always cover the next unfolding leaf, the injury often becomes disastrous to the plant by preventing the new shoot from developing. The heads are first attacked when in blossom, the pollen being eaten greedily. The tender stamens and pistils are lacerated badly and dry up very quickly, so that the embryo seeds are killed in a kind of injury seldom observed and one wherein the damage can hardly be estimated, although evidently it is considerable. As soon as the grains begin to form, the thrips attack the husk, and later, gaining access to the husk, they lacerate the tender integument of the newly forming grain. Grains attacked at this stage are practically destroyed, and even after the milk has become a dough the injury causes the grains to shrivel.

The last portion of a wheat plant to ripen is the head, and therefore the thrips remain on it until it becomes dry. They often stay on the green heads until harvest, but leave the plants very soon after these have been bound up into sheaves, afterwards subsisting on the common grasses present in the fields.

## FIELD OBSERVATIONS

The depredations of this tiny insect were first brought to the writer's notice in the spring of 1908 at Pawnee, Okla., and Wellington, Kans. Here they were first observed in abundance, doing much damage to the new shoots of young growing wheat. With a few sweeps of the insect net they were collected by the thousands from wheat plants throughout April and May. In one instance where they were so very numerous the crop was not worth harvesting, but the failure of the crop could not be attributed entirely to the thrips, owing to the presence in abundance of

both the Hessian fly (*Mayetiola destructor* Say) and the chinch bug (*Blissus leucopterus* Say).

The thrips again appeared in the spring of 1909 in considerable numbers, but not enough to cause noticeable injury. In April and May many eggs and larvæ were killed by the hot sun and wind, on account of the drying of the wheat leaves. However, some of them thrived and reproduced freely, for in August, when volunteer plants sprouted, they occurred in large numbers and continued to reproduce and feed on the fall-sown crops until hibernation.

The winter of 1909–10 was very severe, and only a small number of this species survived; consequently, but few were found during the year 1910 and a still smaller number were noted in 1911 and 1912.

In the late fall of 1912 females were found in clumps of *Agropyron smithii* and also a number were in stools of wheat, in which they hibernated. Thrips were common on wheat and grasses during the growing season of 1913, hibernating in the late fall and appearing in swarms on young wheat in early March, 1914. By the 1st of April the larvæ, now nearly grown, were cutting the shoots severely. They ceased feeding about the third week of April and pupated. By the time the adults were ready to issue, the wheat plants had outgrown all previous injury. Favorable rains produced a rapid growth of wheat, which apparently did not interfere with oviposition or with the feeding of the young thrips larvæ. By the middle of May, when the wheat was heading, the second brood of larvæ readily infested the young heads, feeding upon the stamens, pollen, and pistils, and later attacking the integument of the grain. Larvæ and adult thrips continued to feed on the heads until the crop was harvested, very few being dislodged by the binder. Subsequently they clung to the heads for three or four days, finally leaving the grain for grasses or entering the soil for pupation.

Neither adults nor larvæ could be found in stubble fields during the summer, but as soon as volunteer wheat plants pushed up in early September the thrips were found in all parts of the field, which would indicate that they had been present all the time. As no evidence of thrips was found in the fields during the summer, it follows that the pupæ had waited for the rains, the moisture being sufficient to sprout wheat grains and also to cause the adults to issue.

The thrips did no appreciable injury to young plants in the fall of 1914, but in early December of that year many were hibernating in the principal host plants, the stools of wheat and clumps of *Agropyron smithii*.

## HIBERNATION

The adults and larvæ hibernated in clumps of wheat, *Andropogon scoparius*, and other grasses in the fall of 1909. Although living adults and larvæ were found in wheat on February 8, 1910, and again on March 1,

indicating that they had successfully passed the winter as both adults and larvæ, no eggs could be found on or in wheat leaves.

Both adults and larvæ have been found hibernating beneath the sheaths of the following grasses: *Triticum vulgare* (wheat), *Andropogon scoparius*, *A. furcatus*, *Poa pratensis*, thick mats of *Agropyron smithii*, and *Tripsacum dactyloides*. No stages of thrips have been found hibernating in *Panicum crus-galli* or *Syntherisma sanguinalis*, which become quite dead and dry after the first frost and are abandoned, the thrips continuing to feed on other plants during the warm days that usually follow.

## ENEMIES

Among the more important enemies of *Prosopothrips cognatus* are *Triphleps insidiosus* Say and the larvæ of *Chrysopa oculata* Fab., which consume large numbers. When the thrips are numerous, the fields are literally swarming with the Triphleps. No coccinellid adults or larvæ, not even of the smaller species, have been observed feeding on them, either in the field or in confinement. No parasites have been reared, although it is possible that some parasite materially assisted in reducing their numbers in 1910. No birds have been observed feeding on them.

## CONTROL

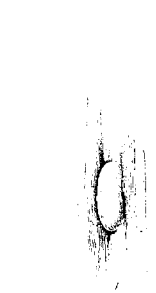
At this time no thoroughly practical remedy can be offered for the control of this pest. Large numbers of thrips may be destroyed by burning off all grasses, but the young wheat fields, where most of the pests are located, can not, for obvious reasons, be burned; nor is it practicable to spray wheat since the expense of the operation would be greater than the returns.

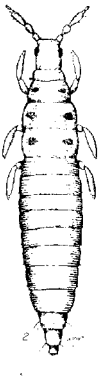
Careful observations on plowing at different times from the middle of June until September seem to favor early plowing, for, although all fields under observation were infested, the two plowed in June and harrowed in late July were attacked to a lesser degree, and these fields contained practically no volunteer wheat.

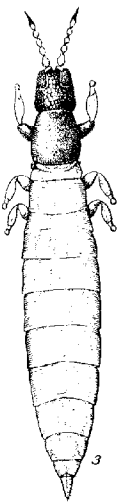
Where this species becomes numerous, it appears that when stubble fields are burned over and plowed early, destroying all grasses, and especially volunteer wheat, there is less opportunity for the thrips to increase in numbers sufficient to damage the crop.

## PLATE XXX

Fig. 1.—*Prosopothrips cognatus:* Egg.
Fig. 2.—*Prosopothrips cognatus:* Larva.
Fig. 3.—*Prosopothrips cognatus:* Adult.
Fig. 4.—Wheat leaves showing injury by *Prosopothrips cognatus.*







# CYTOLOGICAL STUDIES OF AZOTOBACTER CHROOCOCCUM[1]

By AUGUSTO BONAZZI,

*Assistant in Soil Biology, Department of Soils, Ohio Agricultural Experiment Station*

## INTRODUCTION

Because of its advantage to agriculture, the maintenance of a nitrogen balance in the soil has long been studied by scientists. Among the lower organisms involved in this phase of agricultural economy the Azotobacter group, first described by Beijerinck (2)[2] in 1901, is surely one of those that deserve very close study because of their extreme importance.

Since the first work of Berthelot (4) on nitrogen fixation by soil, the Clostridium and Azotobacter groups have been discovered and the methods for their study have been brought to the standard basis, so that these organisms can now be justly compared to any other organism belonging to the class of the Protista. Unfortunately, not till very recently has the cytology of *Azotobacter chroococcum* been studied. But since cytology in all the applications of bacteriology, such as pathological bacteriology and the bacteriology of the tanning, retting, and fermentation industries, has been overlooked by many, it is not surprising that such studies have been deemed unnecessary or unimportant in agriculture.

It is a rational hypothesis very often expressed that every change in physiological activities is accompanied by a like change in the cytological structure of an organism.

Unfortunately, with our present methods, there is no possibility of studying changes in structure, especially if these are small, with that accuracy which it is possible for us to employ in detecting changes in physiological activities.

Furthermore, the majority of methods commonly used in bacteriological studies, especially the staining methods and processes, are only of a diagnostic and medical value, unsuited for refined work. More delicate methods should be used if very slight differences in structure are to be detected.

Although the size of *A. chroococcum* makes the organism ideal for a cytological study, it has been completely ignored for years. Its biological functions play such a great rôle in the nitrogen cycle in nature

[1] Thanks are due to Dr. E. R. Allen, under whose direction this work was undertaken, for kind advice and criticism.

[2] Reference is made by number to "Literature cited," p. 238-239.

under experimental conditions that the question may well be raised whether such functions are equally performed under what might justly be termed "abnormal conditions."

It is ordinarily assumed that the natural condition for *A. chroococcum* is the one in which the organism is obliged to fix nitrogen, since the name "nitrogen fixer" is applied to it. We know now, as a result of considerable work on the part of a number of scientists, that the growth of *A. chroococcum* is stimulated by the presence of small quantities of nitrates, and we know, moreover, that *A. chroococcum* has the power, according to Beijerinck and Van Delden (3), to transform nitrates directly into ammonia, and that, according to Sackett (16, p. 38–39), it forms more of the characteristic pigmentation when grown in a medium containing 0.5 per cent of sodium nitrate than in a medium poor in, or free from, nitrates.

We know also from Sackett (16, pp. 38–39) that ammonium chlorid, ammonium sulphate, asparagin, and peptone do not have the same effect upon this function. The quantity of sodium nitrate used by this author is five times stronger than the one used in ordinary denitrification experiments.

Since this work indicates that a highly oxidized nitrogenous material, such as a nitrate or nitrite, is the only one that seems to accentuate growth and that this same material is attacked and consumed by *A. chroococcum*, are we not justified in believing that the condition of life presenting these compounds as foods might constitute a favorable and perhaps an optimum or normal condition for development? In other words, *A. chroococcum* may be a nitrogen fixer under only such conditions as those which we call "normal"—i. e., when a lack of soluble nitrogenous food is present—and a denitrifier when such conditions are changed—i. e., when there is a possibility for it to consume nitrate under "naturally normal" conditions. It is well known that this micro-organism is a facultative nitrogen fixer, but which is its normal and which its abnormal function is as yet an unsolved problem. A cytological study might possibly throw some light on the question. Appreciable changes in structure, detectable by appropriate methods, might accompany changes in physiological functions. An example of this kind of change is given in the recent work by Nello Mori (13), in which he finds that the cultivation of *Caryobacterium equi* (a pathologic form) on carbohydrate or alcoholic media individualizes in the cells of the organism a so-called nucleus, which was not visible in cells grown on peptone medium.

A study of the change due to environment in a unicellular organism will quite naturally simplify a corresponding study in higher plants, in which there have been so much dissension and contradiction. Before undertaking this study we should determine the nature of the cell constituents which are capable of undergoing changes with variation in

environmental conditions. With the object in mind of clearing up this point, a brief review of the literature on the cytology of *A. chroococcum* is submitted.

## HISTORICAL REVIEW

Beijerinck (2) early described *Azotobacter chroococcum* with regard to its morphology. With the aid of Prof. Zetnow, he determined that the organism was capable of movement by means of a polar cilium. This author describes sarcina-like packets in cultures of *A. chroococcum* and also points to the supposition that these might be resistant forms to replace the spore stadium, which is not present in this organism. These same packets were observed by Krzemieniewski (10, p. 932–941), who found, furthermore, that the cells escaped later from their envelope, leaving it like an empty sheath. Their resistant nature was to some extent doubtful.

A very interesting contribution to the cytology of *A. chroococcum* was made by Prazmowsky (15), who used nearly exclusively vital staining with methylene blue to determine the structure of this organism. He observed the division of what he called "nuclei" (which, according to the description, are Heinze's (8, p. 57) glycogen granules, Ashby's (1) glycogen granules, H. Fischer's (5) volutin granules, and Mencl's (11) nuclei) in the cells of this Azotobacter and stated that the division of this nucleus was followed by the division of the entire cell. By this means were formed chains of elements, which later separated in single individual cells. Prazmowsky denies completely the presence of glycogen in vegetative cells of *A. chroococcum*, but admits its presence in the cells representing the resting stage of the organism—the so-called arthrospores. He holds to the presence in nature of three types of cells of *A. chroococcum:* (1) Nuclear cells (*Kernzellen*), (2) alveolar cells (*alveolare Kernzellen*), and (3) cells with a diffused nucleus (diffuse *Kernzellen*). Some drawings presented by Prazmowsky (15) resemble the sarcina-like packets described by Beijerinck (2) and pictured by Krzemieniewski (10).

Jones (9) holds views completely different from those of all the preceding authors. He considers the granules within the cells of *A. chroococcum* to be of two kinds, one kind, more often found, consisting of glycogen, and the other of a substance that makes up the body of what the author calls "gonidia," which are capable of flowing from the mother cell and are provided with very long delicate cilia.

Mencl, in his work already mentioned (11), used the same staining methods as Prazmowsky (15), and came to the same conclusions as that author.

From this very brief summary of the literature on the subject it is evident that opinions as to the constitution of the cellular make-up of *A. chroococcum* differ widely.

Since the most important constituents of the cell seem to be the granules, a study of their constitution will be first taken up. So many different hypotheses have been presented with regard to their constitution that the following studies were undertaken in order to determine it.

## EXPERIMENTS WITH THE ORGANISM

### CULTURE USED

After having observed the presence of granules in the cells of some cultures of *A. chroococcum*, microchemical studies were undertaken to determine their nature and to ascertain whether they could be stained in such a way as to be easily individualized in future studies. Some microscopical preparations had already shown that the Guignard (6, p. 15) method was the one to be depended upon, but to study this point better, a series of slides were prepared and stained from an old stock culture that had been cultivated on ordinary mannit agar for two years without having at any time been rejuvenated in soil. The culture had apparently lost the power to produce pigment, as it had not produced any for the last five or six transfers on solid medium. This culture was used in all the experiments described in this paper.

The growth on mannit agar was white, transparent, strongly raised at the beginning, flattening with age, smooth, soft, and invading the slant at the bottom.

The culture had been forwarded to the Ohio Station by the Office of Soil Bacteriology and Plant Nutrition, United States Department of Agriculture, and had been obtained by that institution from the American Museum of Natural History. At the time it was received in this laboratory it bore the label "*Azotobacter chroococcum* Beij." and has been kept ever since in pure culture.

When cultivated on mannit agar, to which had been added 1 gm. of potassium nitrate per liter, the culture regained its pigment-producing power, assuming a waxy and glossy appearance.

Since the organisms vary from cocci forms to bacillary ones, no single measure can be given to the species, but a distinction must be drawn between these two forms. The cocci forms measured 1 to 2$\mu$ in diameter and the bacillary forms 3 to 4$\mu$ by 1.5 to 2$\mu$. The nitrogen-fixing power of the organism was very slight, since it fixed only 1.26 mg. of nitrogen in 25 c. c. of solution in 37 days in pure culture.

### STAINING THE ORGANISM

Many methods of staining the organism were tried in the hope of finding one that would be adapted to follow up the growth and rôle of the granules in the metabolism of the cell. All the methods used gave satisfactory results. The solutions were tested several times on blood as a

standard stainable substance. Since the nature of the components of polymorphonuclear leucocytes is known and since the solutions responded alike on these and on the cells of *A. chroococcum*, we are justified in comparing these bacterial cells with the blood cells.

GUIGNARD'S METHOD was originated by A. Guignard (6, p. 19), who used it to study the cytology of antherozoids. The organism was fixed with osmic-acid fumes and stained in a mixture of 50 c. c. of a solution of 2 per cent of fuchsin in 1 per cent of acetic acid, 40 c. c. of a solution of 0.2 per cent of methyl green in 1 per cent of acetic acid, and 1 c. c. of acetic acid. This method gave a splendid picture of the structure of the cell. The network, of which mention will be made later on, was stained violet, the contents of the network were hardly stained at all, and the granules were a deep violet-black.

The Guignard (6, p. 19) method did not give the color differentiations desired, red and green, perhaps on account of metachromacy of the methyl green used in the solution. On the whole, it proved to be a very satisfactory method. Plate XXXI, figures 2, 3, 4, 6, 8, and Plate XXXII show some very good preparations obtained by it; the network showed plainly and the granulations very distinctly. Plate XXXIII, figure 1, shows some cells stained by this method; several granules are to be seen. The cell wall is quite evident in old cells, but in young ones it is to be seen only slightly stained.

HEIDENHAIN'S ORDINARY FERRIC HÆMATOXYLIN stained the network strongly, but gave no differentiation. The fixing was done by passing the glass through a flame.

The Heidenhain method was used progressively in a great number of cases and regressively only occasionally. When progressive, it stained the cell components black, and it demonstrated the zooglea sheaths, such as Beijerinck (2) and Krzemieniewski (10) found. Plate XXXI, figure 5, shows some cells obtained by the Heidenhain method. Plate XXXIII, figure 3, shows some zooglea and cells escaping from them, and Plate XXXIII, figure 2, shows two of these zooglea nearly empty, all cells having escaped, the same as is shown in Plate XXXI, figure 5. In some groups the walls which formerly separated the different cells in the same mass are to be seen quite distinctly after the cells have escaped (Pl. XXXI, fig. 5).

Smears from a hay-agar culture of *A. chroococcum* stained by the regressive method showed the network to be made up in many cases of a substance taking a beautiful dark-violet color and of a nearly transparent appearance.

ROMANOWSKY'S SIMPLE STAIN is the one used by the originator for the study of the parasitology of malaria. The preparation was fixed by the flame and stained in a mixture of five volumes of eosin in a 1 per cent aqueous solution and two volumes of a saturated aqueous solution of

methylene blue. This method gave nearly as good results as Guignard's *(6, p. 19), but no color differentiation was obtained.*

*The Romanowsky simple method was not the most satisfactory, on account of the lack of color differentiation, although it demonstrated quite clearly the cell structures.*

METHYLENE BLUE, 1 to 1,000, in aqueous solution (flame-fixed), gave good results; but, although repeated examinations were made of the slides thus prepared, no red color was visible, the granules being indicated only by a strong black-blue color.

ROMANOWSKY'S COMPOUND STAIN (after Harris). No fixing was done, since the methyl alcohol itself served as a fixing agent. Staining was done by the mixture of methylene azure and methylene violet and with eosin and methylene blue in methyl alcohol. This method, although very good in showing the structure of the cells, failed in most cases to give sharp differentiation. No real red color for chromatin was obtained, as the one that would result from the staining of the hæmoparasitic protozoa. Some human blood stained by this solution gave the usual color presentations. Leucocytes of all kinds, polymorphonuclear, macronuclear, and eosinophylic, were seen, stained in their characteristic and distinct colors. When *A. chroococcum* was stained by this method, the network took a blue color and the interior of the meshes a pink one. Repeated trials with the methylene-blue and glycerin method as used by Mencl (11) failed to give satisfactory results; the cell always took the blue, and the granules never took the red color that Mencl ascribes to them. Vital stained cells present the same structure as the one described by Mencl (Pl. XXXI, fig. 1).

In the Romanowsky-compound method great attention must be paid in diluting on the cover glass, since the solution tends to flow on the underside and make a miscroscopical examination difficult. The time of action for maximum differentiation varies with different objects. After several trials the time limit for the action of the stain in these tests was fixed at 10 minutes. After the addition of water to the stain on the cover glass, the washing should be very rapid. The best preparations were obtained when the washing was not prolonged over half a minute. Distilled water was preferred in the washing.

The cells of *A. chroococcum* stained differentially; the network took on a blue color, while the contents of the network meshes took on a pink color. If the washing is prolonged, the pink color disappears and only the blue remains in the cell, as in blood stained by this method.

### MICROCHEMICAL STUDIES

Since the work of Mencl (11), Jones (9), Prazmowsky (15), and others has shown that the structure of the cell of *A. chroococcum* is complex, the present writer next proceeded to determine microchemically the nature of the different components.

The methods used are those proposed by A. Meyer (12) and by his follower Grimme, and are already recognized as valuable in the study of the cytology of bacteria.

The granules observed by the writer in the cells of this organism might be nuclei, metachromatic granules, fat drops, glycogen, or starch.

Mencl (11) and Prazmowsky (15) believe them to be nuclei or nucleo equivalents; Jones (9), glycogen granules and nuclei of gonidia; and Fischer (5), metachromatic bodies. None of these authors has attempted to prove his point satisfactorily or to disprove other possibilities.

Since the aim of this part of the work is to find the true nature of the granules met with in the organism in question, the methods used will be described and the results of the investigations given.

STARCH.—Smears from cultures on mannit agar, flame-fixed, were mounted in a saturated aqueous solution of iodin, sealed with paraffin, and observed at once and 24 hours later.

The granules are not to be considered as starch, since they do not give a blue color to iodin or to Meissner's solution.[1]

Even after 24 hours no coloration was visible. The cell diaphanized, and the granules gained in refraction.

FAT.—Smears from a mannit-agar culture were immersed in ethylic ether for 1¼ hours, dried, stained in methylene blue, and mounted in balsam.

Since A. Meyer (12) claims that chloroform and alcohol are not good solvents of bacterial fats, on account of the difficulty which they meet in passing through the cell wall and *Schleimschicht*, the writer tried to avoid any objection that could be raised against the conclusions drawn from the results obtained by the ethylic-ether method.

With this aim in view tests were made, using the method suggested and recommended by Meyer. It consists in fixing the bacterial smear in formol, immersing in glacial acetic acid, neutralizing, and staining. Methylene blue, 1 to 10, was used as the stain; it gave very good preparations. The fats are dissolved by this method. Treatment with glacial acetic acid did not dissolve the granules.

Prolonged treatment in the cold with ether or with glacial acetic acid did not dissolve the granules, since those which had been treated stained just as strongly as the checks. This eliminates the question of their fatty nature, although their deeply staining property should have already led to this supposition. It should be noted that these preparations were not fixed in osmic acid, which treatment would render lipoids, or fatty substances in general, insoluble in fat solvents, and stainable by the ordinary staining solutions. For this reason their myelin nature should not be accepted.

[1] Formula for Meissner's solution: Metallic iodin, 7 gm.; potassium iodid, 20 gm.; water, 100 gm.

GLYCOGEN.—Smears were mounted in a saturated aqueous solution of iodin, sealed with paraffin, and observed at once or 24 hours later.

Meissner's solution instead of iodin was also used.

A. Meyer (12) also suggests boiling for three minutes in a weak solution of sulphuric acid (2 drops of concentrated $H_2SO_4$ in 5 c. c. of $H_2O$). Glycogen is dissolved by this treatment.

The granules did not give any golden color with iodin solution, even after 24 hours, contact. Meissner's solution, not removed from cells, gave a very dark golden-yellow color. To be certain, however, of the presence of glycogen in the cells, this golden color should persist after the excess of Meissner's solution has been replaced by water by means of capillarity. The preparations of the writer, nevertheless, did not retain the golden coloration after capillary washing of the mount, although a strip of filter paper placed on the edge of the cover glass in contact with the wash solution took on a faint-blue color.

The granules were dissolved by the treatment with sulphuric acid. It is to be remembered that also metachromatin is dissolved by boiling in water for three to five minutes. If the sulphuric acid in the cold were not to dissolve the latter, perhaps it would at the boiling temperature. Moreover, the sulphuric-acid solution used for testing the solubility of metachromatin is 1.2 to 1.3 times stronger than the one used for the detection of glycogen by this method, which would probably account for the solution of the granules. The granules in this case, according to my view, are not of a glycogenous nature.

The mounting of smears in a dilute Meissner solution (2 drops in 5 c. c. of $H_2O$) showed the cells stained in a homogenous manner straw-yellow, while the mounting of a smear of a blastomycete (*Saccharomyces cerevisiae*) in the same solution gave a decided golden-brown granulation.

METACHROMATIC AND CHROMATIC GRANULES.—To distinguish between the two kinds of granules, several tests were used.

(*a*) The ruby-red color, which should be developed by the Romanowsky-compound method, indicates chromatin. Protozoa are a good example of the results to be obtained.

In the large number of slides prepared no ruby color was developed by the Romanowsky stain.

(*b*) A cover-glass preparation was stained with methylene blue, 1 to 11. After washing in water treated with 1 per cent of sulphuric acid, chromatin should discolor at once, while metachromatin should not.

Treatment with the sulphuric-acid solution did not decolorize the granules, but it decolorized the cell network.

(*c*) A cover-glass preparation stained with methylene blue was treated with a 5 per cent solution of sodium carbonate. Chromatin should remain colored; metachromatin should discolor.

Treatment with the sodium-carbonate solution discolors the granules, leaving the cell network unchanged.

(*d*) A cover-glass preparation stained with methylene blue was treated with Meissner's solution and then with a 5 per cent solution of sodium carbonate. After treatment with the Meissner solution the cell should be brown and the granules decolorize if of metachromatic nature; they should remain colored if of chromatic nature.

This treatment left the granules decolorized and cells stained blue.

(*e*) On treating with boiling water and then staining or boiling in a test tube for two minutes and then staining, the metachromatin should dissolve.

Boiling with water over Bunsen burner for two minutes completely dissolved the granules. Washing with water at 90° to 99° C. for four minutes did not dissolve all the granules, perhaps on account of insufficient temperature; thus, only some granules, the large ones much reduced in size, are to be seen after this treatment. The contents of the network took a light-pink color when treated with methylene blue, 1 to 11.

(*f*) Smears were prepared and stained by the Ernst method. Blue coloration of granules after the Bismarck-brown treatment would therefore indicate metachromatin.

This method gave a bluish color to the granules, but these preparations do not last when mounted in balsam (dissolved in chloroform).

(*g*) On staining unfixed preparations with methyl green the dye should stain only chromatin.

Staining with methyl green showed a strong metachromacy, since the granules took on a violet color. The staining of unfixed preparations in solutions of Grubler's methyl green gave a violet color to the granules. Later, in trying to explain this metachromacy, tests were made on the purity of the stain. They showed it to contain considerable impurities in the form of violet dyes, probably methyl violet.

Mounts in 5.*N* potassium hydroxid and in calcium chlorid gave preparations that did not especially change in appearance from the checks.

### RESULTS OF MICROCHEMICAL TESTS

From what has been said about the granules found in the cells it may be stated that their nature seems to be different from that of the granules found by Mencl (11), Jones (9), Prazmowsky (15), and others in the organism on which they were working, with the exception of those treated by Fischer (5).

In fact, as has been seen, the granules found by the writer did not give the reaction for glycogen. The results obtained with Meissner's solution must be carefully judged before making a definite statement. If the granules were glycogenic, they would not only give a golden color when treated with the reagent but would also retain it if the excess of the

reagent were extracted, just as filter paper would if treated with the same solution and then an excess of the reagent washed off.

The color might disappear if the washing were very prolonged, but in this writer's case the washing was not thorough, because when the cover glass was sealed to the slide a strip of white filter paper, brought in contact with the solution, gave a bluish color, indicating presence of a reagent. Moreover, as Meyer (12) has shown, a very small amount of a reagent is necessary to give reliable results. Besides, treatment with a concentrated solution of iodin for 24 hours gave no trace of coloring whatever to the granules. Treatment with Meissner's concentrated solution of cells of the organism, kindly forwarded us by Prof. D. H. Jones, gave a strong golden-brown color.[1]

It should be observed that the trials were repeated also on old cultures (14 or 15 days old), but no reaction took place. This would be contrary to the statement by Jones (9) to the effect that the cells give less glycogen reaction when young than when old. Also in one single slide of a comparatively young culture there ought to be many fully developed cells that should react. It therefore appears natural that the granules observed by the writer should be classed among those which, according to Jones (9), "do not always appear to be present, do not give the glycogen reaction, but do stain with various aniline dyes."[2] Their failure to take the blue color with iodin excludes their starchy nature. They are not fats, because treatment with ether did not dissolve them, because they stained with extraordinary facility, and also because they were not dissolved by treatment with glacial acetic acid. It is very probable that the granules observed by Mencl (11), which stained easily with methylene blue, are the same as those of Jones (9), just quoted.

Although repeated trials were made to obtain the color differentiation reported by Mencl (11) with methylene blue and glycerin, the results were always unsatisfactory. The granules never stained red.

Mencl (11) and Prazmowsky (15) class the granules as "nuclei- or nucleo-equivalents," thus implying that they have a chromatic nature. Jones (9) considers them as motile flagellated granules, resembling the reproductive organs of many Cyanophyceae. This naturally implies that they contain chromatin as a necessary constituent. Neither of these authors proved his assertions with standard methods.

The granules that were obtained by the writer stained easily and deeply, but gave no reddish color with a 1 to 1,000 methylene-blue solution, though they gave it with a 1 to 5,000 solution, as metachromatic granules usually do. They responded positively to all the tests carried out to distinguish their metachromatic character.

[1] Since this method is not reliable, the present writer intends, in due time, to make a quantitative determination of the glycogen to be found in large quantities of cells of *A. chroococcum* grown under conditions equal to the preceding.

[2] As to the ciliated gonidia described by Jones (9), see under "Staining the organism" and "Discussion of results."

A summary of the results of the tests to distinguish between metachromatic and chromatic granules is given in Table I.

TABLE I.—*Summary of results of the tests for metachromatic and chromatic granules*[a]

| Treatment. | Theoretical results: metachromatin. | Actual experimental results: granules. |
|---|---|---|
| (b) Methylene blue (1:10)[b] and 1 per cent of sulphuric acid | + | + |
| (c) Methylene blue (1:10)[b] and 5 per cent of sodium carbonate | − | − |
| (d) Methylene blue (1:10),[b] Meissner's solution, and 5 per cent of sodium carbonate | − | − |
| (e) Boiling for 2 minutes in water | − | − |
| (f) Ernst method, blue color for metachromatin ("volutin") | + | + |
| Weak methylene-blue solution shows red color | + | + |

[a] + signifies a coloration; −, a discoloration.
[b] Methylene blue (1:10) = 1 part of methylene-blue saturated solution added to 10 parts of water.

As can be seen from Table I, there is no doubt that metachromatic or, as A. Meyer (12) terms them, "volutin" granules were found.

## DISCUSSION OF RESULTS OF THE EXPERIMENTS

Since, according to the tests performed, the metachromatic nature of the granules is assured, their action in the cell will be studied to ascertain whether they have a rôle in the metabolism which confirms the microchemical investigations.

As we know, Meyer (12) considered metachromatic granules as reserve foods, while MacCallum and Carlier, according to Guilliermond (7, p. 199), considered them as "zymogenic granules." Although the writer does not believe a conclusion as to whether the granules of the writer are reserves or zymogens can be reached in the present paper, he thinks that by presenting some points of the question a working hypothesis may be sketched for future work.

One of the main points for consideration is the disposition of these granules in the cell—i. e., to determine whether a regular disposition takes place at some time in the life of the cell or whether the granules have no special setting in the organized unit.

To begin with, the drawings furnished by Mencl (11) will be studied. In nearly all cases in which a reticulated structure is to be seen, the granules are placed on the juncture of the net meshes or also in the center of the meshes themselves. As to the regularity of distribution in the cell, these granules present none, because, as Mencl himself states, all stadia, from fine scattered points to large globules, are to be found. Considering the large globules as the maximum development of the material composing them, the most advanced stage morphologically, they should be expected to occupy a uniform place in the fully developed

cell. From Prazmowsky's work (15) nothing can be decided with regard to this point. This author does not give much importance to it, and his drawings show the same thing as those of Mencl.

Might not a regularity in the setting of the granules, not determined by the above-named authors because of unappropriate staining methods, be presented at some time during the life of the cell?

To determine this point it is necessary to know just what are the changes which the cells of *A. chroococcum* undergo with age. These queries will have to be solved in the following order:

(I) What are the changes in the cytology of *A. chroococcum* at different ages of the same cell?

(II) What is the relation of the changes in cytology to the fate of the granules of the cell?

Since difficulties in operative technique make the solution of Question I impossible,[1] its wording must be changed to the following: (I) What are the cytological changes undergone with age by the cells of *A. chroococcum?* Plate XXXI, figures 6 and 7, gives a graphical solution to this question. A few words must be said about the stadia which were found in the life cycle of the organized units.

That the reticulated structure found in the cells in the writer's tests was not due to the drying before fixing or to the fixing is proved by the fact that many observations of vital-stained preparations made in this laboratory and also by other authors showed the same structures as did the treated cells. Moreover, the different methods of fixing used, osmic-acid fumes, methyl alcohol, and flame—i. e., gas, liquid, and heat—all gave the same structures. Therefore these structures can not in any degree whatsoever be considered as artifacts produced by the fixing agents.

The first stadia were found with undifferentiated cytoplasm and metachromatic granules (Pl. XXXI, fig. 6, No. 1; fig. 7, No. 1, 2, 3).

Second, a stadium of cytoplasmic differentiation or reticulation was found in which the cytoplasma contracts toward the sides of the cell, leaving some strands to connect the accumulations of cytoplasma placed in different places of the periphery (Pl. XXXI, fig. 6, No. 2–10; fig. 7, No. 4–7).

Third, a division stage was found, in which the cells after elongation and differentiation in peripherical and transversal cytoplasma divide and form a wall between the resulting daughter cells. The second and last stadia are interdependent.

Such a succession of stadia would then be in conformity with the one resulting from the studies on *Bacillus anthracis* by Pénau (14). The nuclear phase was not found in the organisms in the experiments of the

[1] Vital staining, although very valuable in following cell division, could hardly give here results to answer the question. Prazmowsky's (15) and Mencl's (11) interpretations show the disadvantages of the method.

present writer; perhaps because he did not use the same methods as Pénau. As can be seen from the accompanying illustration (Pl. XXXI, fig. 6, 7) the granules do not disappear from the cells at any time of the life cycle of the latter; neither do they show any uniform setting. Possibly they would correspond in *A. chroococcum*, to the so-called nuclei of *B. anthracis* if they did not exist already in the undifferentiated stadium and if they did not persist in the reticulated stadium. As Pénau states, the metachromatic granules already exist in the undifferentiated cells of *B. anthracis* and persist in the reticulated cells, while the structure which he calls true nucleus disappears at the outset.

In Plate XXXI, figure 6, No. 6, and in Plate XXXII, it can be seen that the granules may even be placed on the outside of the cell. Mencl (11) also has noted this fact, but does not attempt to give any explanation in regard to it. The granules increase in size with age, but, aside from this character, they present no other.

Their plurality could not by itself exclude their nuclear nature, but that character in addition to their occasional extracellular position would be sufficient to deny to them nuclear functions. This point was not considered by Mencl as one worthy of attention.

How could he consider these granules, escaping from the cells as nuclei, as the most important and most vital organs of a cell? It is true that in higher plants some cells (sieve tubes) are also left in old age without nuclei, but it is also true that in most cases these cells are no longer capable of reproduction. These granules have also been found to escape from cells which were undergoing the process of division. No isolated mass of cytoplasm has ever been seen to divide spontaneously.

Plate XXXI, figures 8 and 8a, shows some other cells stained by the Guignard (6) method. All stages are here represented.

Some cells, which are easily found, do not contain any granules, although their size indicates an advanced age. This lack of granules might possibly be attributed to an expulsion of the same by the method just mentioned.

To judge from Mencl's (11) drawings, many cells present one granule, but no regularity as to its setting in them. In Plate XXXII, which represents some cells drawn at random from a Guignard-stained preparation, the granules are always seen embedded in the cytoplasmic matrix [1] and are never to be seen inside the meshes of the cell network. Sometimes

[1] The expressions "cytoplasmic matrix," "cell network," "cytoplasmic strands" are here used to mean that part of the cell contents that in our organisms has an affinity for basic dyes.

As we have already seen, the Romanowsky compound stain gives a differentiation of colors in the cells, the network taking the blue color characteristic of nuclei and the contents of the meshes taking a pink color. Some comparative preparations with normal and abnormal human blood were studied; the same staining solution as the one used for *A. chroococcum* gave the ordinary colors.

Nothing should now prevent the naming of the basophylic cell constituents "nucleo substances," and the cosinophylic ones "cytoplasm." But since we mean to furnish more proofs to establish their nature, we will continue to use the terms "matrix," "network," "strands," according to their morphology. See also Plate XXXI, figures 2, 3, and others.

at first glance they seem to be completely detached from the cytoplasmic network, but a closer observation shows very feebly staining joining strands. Preparations from liquid cultures also present the same cellular structures.

## CONCLUSIONS

From the present paper the following conclusions may be drawn:

(1) The cells of *Azotobacter chroococcum* Beij. present a complex nature and different stadia of cytological make-up. Conforming to the conclusions of Pénau (14) on *B. anthracis* and all endosporous bacteria, *A. chroococcum* shows an undifferentiated stadium. The nuclear stadium and the sporogenous one were not studied in the present paper.

(2) The organism with which we are working presents peculiar granulations, which seem not to have any relation to the reproduction of the cell.

(3) These granulations take the basic dyes and are constituted neither of fats nor glycogen, starch nor chromatin. They seem to be of a metachromatic nature.

(4) They seem to have their genesis from the nucleus, since they are always to be found embedded in that part of the protoplasm which shows nuclear characteristics.

(5) Their disposition in the cells is not regular, but changes in different individuals.

(6) Their place in Meyer's (12) system is uncertain, since by the present work on their nature they seem to belong to the class of ergastic structures, or stored material, while according to Prazmowsky's (15) work their reproduction might place them in the class which Meyer calls "protoplastic." Their regular appearance in the cells of *A. chroococcum* might be caused by the special conditions of life.

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## PLATE XXXI

*Azotobacter chroococcum*

Fig. 1.—Vital-stained preparation 37 days old.

Fig. 2.—An 18-hour-old culture stained by the Guignard stain, showing strongly dyed protoplasmic granules.

Fig. 3.—A 65-hour-old culture stained by the Guignard method.

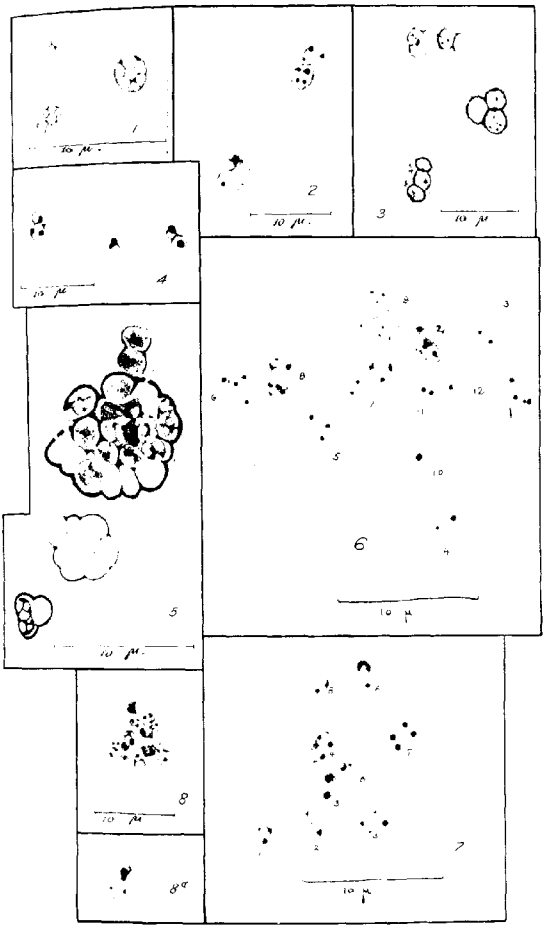
Fig. 4.—A 9-day-old culture stained by the Guignard method. The cytoplasmic matrix is here distinctly visible.

Fig. 5.—A 65-hour-old culture stained progressively by the Heidenhain method showing some empty sheaths of the peculiar zooglea masses detected by Beijerinck and Krzemieniewsky. The partition walls are visible.

Fig. 6.—An 18-hour-old culture on mannit agar, showing the life cycle of the organism. Stained by the Guignard method.

Fig. 7.—An 18-hour-old culture on mannit agar, showing the life cycle of the organism. Stained with methylene blue, 1 to 1,000.

Fig. 8 and 8a.—Cells drawn from an 18-hour-old culture on mannit agar. Stained by the Guignard method.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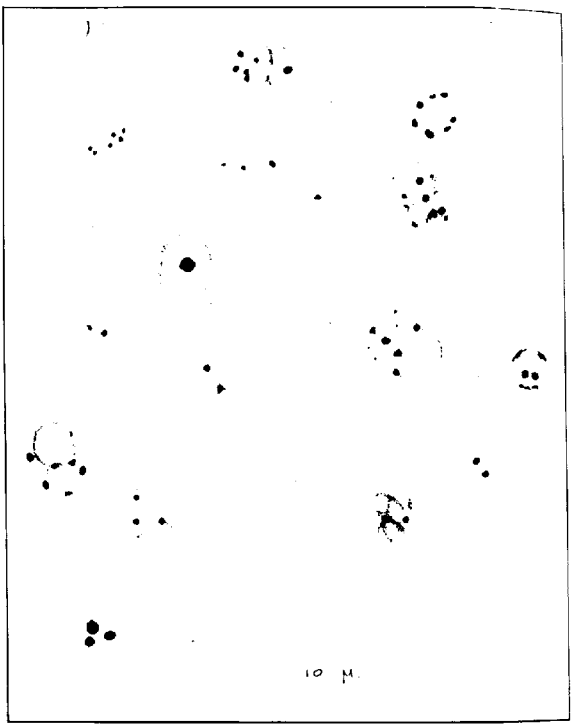

10 M.

PLATE XXXII

*Azotobacter chroococcum:* Cells drawn at random from a 65-hour-old culture on mannit agar. Stained by the Guignard method.

91007°—15——4

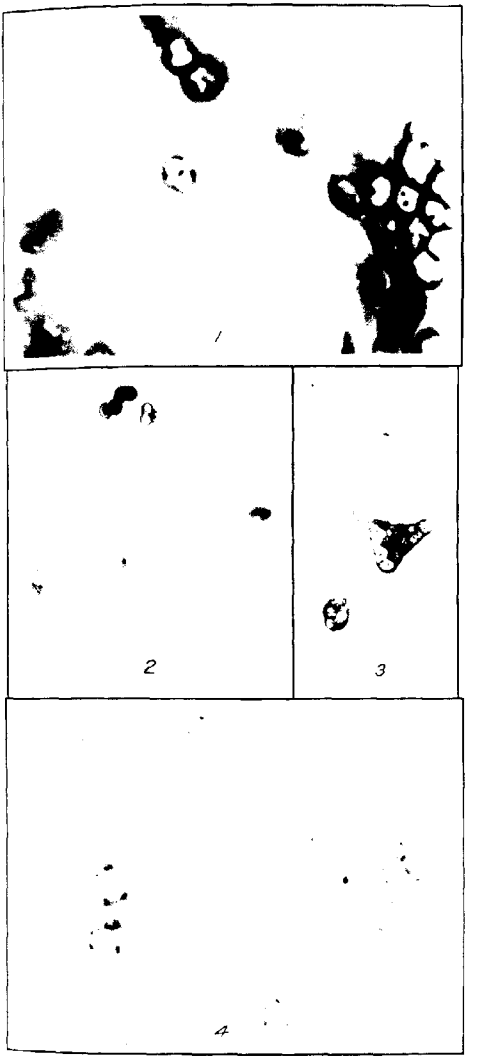
## PLATE XXXIII

*Azotobacter chroococcum*

Fig. 1.—Photomicrograph of a 65-hour-old culture stained by the Guignard method.

Fig. 2, 3.—Photomicrograph of a 65-hour-old culture stained by the Heidenhain method.

Fig. 4.—Photomicrograph of a 2-day-old culture stained with methylene blue, 1 to 1,000.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# INFLUENCE OF SOIL MOISTURE UPON THE RATE OF INCREASE IN SUGAR-BEET ROOT-LOUSE COLONIES

By J. R. PARKER,

*Assistant Entomologist, Montana Agricultural Experiment Station*

## INTRODUCTION

In a study of the sugar-beet root louse (*Pemphigus betae* Doane) carried on by the Montana Agricultural Experiment Station as an Adams project since 1909, it was early recognized that soil moisture was an important factor in controlling the rate of increase in root-louse colonies. By means of general field observations, insectary experiments, and field tests a considerable mass of data concerning this point has been collected; and, because of their bearing on methods of control, they are now published.

## GENERAL FIELD OBSERVATIONS

Outside of the sugar-beet (*Beta vulgaris*) fields the subterranean form of the root louse is most commonly found upon lamb's-quarters (*Chenopodium album* L.) growing in dry situations. The largest and most flourishing colonies are to be found where this weed has pushed its way through a ground covering of dry barnyard manure, pine needles, or other material which provides a comparatively dry medium for the ramification of the smaller rootlets. Lamb's-quarters growing in continuously damp situations makes a larger and more succulent growth, but is rarely heavily infested.

In the sugar-beet fields it has been noted many times that root lice are most abundant and that root-louse injury first appears where the soil is the driest. A striking illustration of this was noticed in a large sugar-beet field that was cut diagonally by a depression in which the ground remained moist without irrigation the entire summer. When the field was visited in October, there was a sharp line of demarcation between the moist soil of the depression and that of the general level of the beet field, which at that time was quite dry. Sugar beets were making a fine growth in the moist soil and not a root louse could be found upon them, while in the drier soil around the borders of the depression nearly every sugar beet was very heavily infested.

## INSECTARY EXPERIMENTS

In June, 1911, insectary experiments were begun for the purpose of determining the influence of moisture upon the rate of increase in sugar-beet root-louse colonies.

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Sugar-beet plants 3 inches in height were transplanted singly into 8-inch pots. After they had started to make a new growth, the earth was pushed away from a portion of the taproot and eight small lice in the first instar and two adults were placed upon each plant. Thirty plants were infested in this manner.

They were divided into three lots and watered as follows: Lot 1, subirrigated every other day from June 15 to August 15; lot 2, subirrigated every day from June 15 to August 15; lot 3, watered from above every day from June 15 to August 15. So far as practicable, the water applied any one day was the same in amount for all plants, but, because of the unequal moisture-retaining capacity of the soil in different pots, this was not always advisable. In general, no plants were kept wet enough to seriously retard their growth, and none were allowed to suffer for lack of moisture. All of the pots were set in large saucers, and, where the plants were subirrigated, the water was poured into the saucer and taken up by the plant as needed. On August 15 the soil in each pot was minutely examined, and all root lice that could be recovered were counted.

The data obtained are shown in Table I.

TABLE I.—*Record of sugar-beet root-louse increase under different soil-moisture conditions. First insectary experiment*

| Number of plants and moisture conditions. | Initial infestation. | Total infestation at end of 2 months. |
|---|---|---|
| 10 plants subirrigated every other day | 100 lice, 10 per plant | 4,554 |
| 10 plants subirrigated every day | ....do | 1,760 |
| 10 plants watered from above every day | ....do | 214 |

Upon plants subirrigated every day 100 root lice increased to 1,760 in two months. Upon plants receiving the same amount of water from above 100 root lice increased to only 214 in the same length of time. This decided difference was probably due to the fact that in the subirrigated pots the soil was comparatively dry to a depth of several inches below the surface. It was from this drier soil that most of the root lice were recovered. Where the water was added from above, the soil was soaked throughout each day, apparently bringing about conditions very unfavorable to root-louse increase.

Upon plants subirrigated every other day 100 root lice increased to 4,554 in two months. By far the greatest number of lice was produced upon the sugar beets grown under the driest conditions.

In January, 1912, practically the same experiment was repeated, the only difference being that instead of applying a certain amount of water on certain days water was applied whenever necessary to maintain the soil conditions desired. Thirty plants, infested as before, were divided

into three lots and watered as follows: Lot 1, subirrigated so as to keep the top 2 inches of soil dry; lot 2, subirrigated so as to keep surface soil slightly moist; lot 3, watered from above so as to keep soil very moist throughout. At the end of two months all root lice that could be recovered were counted. The results are given in Table II.

TABLE II.—*Record of sugar-beet root-louse increase under different soil-moisture conditions. Second insectary experiment*

| Number of plants and moisture conditions. | Initial infestation. | Total infestation at end of 2 months. |
|---|---|---|
| 10 plants subirrigated; 2 inches of dry soil at top | 100 lice, 10 per plant | 7,027 |
| 10 plants subirrigated; surface soil kept slightly moist. | .....do..... | 750 |
| 10 plants watered from above; soil kept very moist throughout. | .....do..... | 211 |

In this experiment root lice living upon sugar beets in the driest soil again showed the highest rate of increase, 100 increasing to 7,027 in two months.

Combining the data from the two insectary experiments, the following statement may be formulated:

200 sugar-beet root lice in rather dry soil increased in two months to 11,581.
200 sugar-beet root lice in rather moist soil increased in two months to 2,510.
200 sugar-beet root lice in very moist soil increased in two months to 405.

## FIELD EXPERIMENTS IN MONTANA

During the summer of 1914 experiments for the purpose of determining the influence of irrigation upon the reproductive power of the sugar-beet root louse were carried on at the following places in Montana: Huntley Experimental Farm, Huntley, Yellowstone Valley; Montana Experiment Station farm, Bozeman, Gallatin Valley; Billings Sugar Co.'s experimental farm, Edgar, Clark Fork Valley. Very different conditions prevailed at each place, and the experiments are therefore reported under separate heads.

### IRRIGATION EXPERIMENT AT HUNTLEY

Six one-tenth-acre plots of sugar beets located on the experimental farm of the Bureau of Plant Industry at Huntley were used in the experiment. Mr. Dan Hansen, farm superintendent, was directly in charge of the experiment, and his hearty cooperation at all times is here acknowledged. The plots were about 3 miles from the nearest cottonwood trees (*Populus* spp.) and were, therefore, not in a position to become so heavily infested as were the plots at Edgar, which were bordered by a cottonwood grove.

The months of July and August were very dry, thereby making it possible to maintain the desired moisture conditions in both the wet and the dry plots. The rainfall in inches from June 15 to September 15 is given in Table III.

TABLE III.—*Record of rainfall at Huntley, Mont., from June 15 to Sept. 15, 1912*

| Date. | Precipitation. | Date. | Precipitation. |
|---|---|---|---|
| | *Inches.* | | *Inches.* |
| June 16 | 0.25 | July 10 | 0.04 |
| 19 | .22 | 11 | .01 |
| 20 | .02 | Total | .05 |
| 23 | .02 | | |
| 25 | 1.23 | Sept. 12 | .62 |
| 26 | .12 | Total | .62 |
| 27 | .33 | | |
| Total | 2.19 | Total for season | 2.86 |

The sugar beets were grown according to ordinary practice, except in the matter of irrigation. Three plots were irrigated, for the purpose of keeping the soil fairly moist at all times. To accomplish this, water was applied five times: July 3, July 10, July 18, July 30, and August 24. Cultivations were given as follows: June 10, June 20, July 16, and July 25. Alternating with these plots were three which were allowed to become quite dry between irrigations; however, they suffered no more from lack of moisture than do many beets grown under ordinary farm practice. The dry plots were irrigated twice: July 22 and August 20. Cultivations were given on June 10 and July 2.

Beginning on September 17 each beet was examined for root lice and its condition recorded as it was pulled from the ground. Beets bearing from 1 to approximately 25 lice were classed as "slightly infested"; beets more than slightly infested but having no more than half of their surface covered with root lice and their waxy secretions were classed as "badly infested"; beets more than half covered were classed as "very badly infested." Badly and very badly infested sugar beets were considered as injuriously infested. A record was also made of the sugar content and the yield in pounds. The sugar analyses were obtained from samples sent to the factory according to the routine method.

The combined results from the six plots are given in Table IV.

At harvest time there was little difference in the appearance of the beet foliage on the various plots, and one not familiar with the experiment could not have distinguished between the plots which received five irrigations and those that received only two. However, an examination of the roots showed a considerable difference in the percentage of beets infested, as well as a difference in sugar content and weight. Under the drier conditions the infestation was 64.7 per cent, while where the soil

was kept moist the infestation was reduced to 31.4 per cent. Moreover, the sugar beets that received the greater number of irrigations yielded the highest in sugar and in weight.

TABLE IV.—*Record of sugar-beet root-louse increase under different soil-moisture conditions. Huntley irrigation experiment*

| Number of plots and number of irrigations. | Condition of sugar beets at harvest. | | | | | | Average sugar content. | Total yield. |
|---|---|---|---|---|---|---|---|---|
| | Number of plants uninfested. | Number of plants slightly infested. | Number of plants badly infested. | Number of plants very badly infested. | Percentage of infestation. | Percentage injuriously infested. | | |
| | | | | | | | *Per cent.* | *Pounds.* |
| 3 plots, 2 irrigations | 2,659 | 4,343 | 457 | 87 | 64.7 | 7.2 | 14.3 | 7,405 |
| 3 plots, 5 irrigations | 5,196 | 2,053 | 245 | 84 | 31.4 | 4.3 | 16.4 | 8,984 |

IRRIGATION EXPERIMENTS AT BOZEMAN

Four one-quarter-acre plots of sugar beets located on the Montana Experiment Station farm were used for the experiment. There were numerous cottonwoods within a mile of the plots, and two years previously sugar beets in the same location had been heavily infested with lice.

The sugar beets were not harvested until October 18 and were subjected to considerable rain during September and early October. The precipitation was considerably greater than at Huntley and Edgar (Table V).

TABLE V.—*Record of rainfall at Bozeman, Mont., from June 15 to Oct. 15, 1914*

| Date. | Precipitation. | Date. | Precipitation. | Date. | Precipitation. | Date. | Precipitation. |
|---|---|---|---|---|---|---|---|
| | *Inches.* | | *Inches.* | | *Inches.* | | *Inches.* |
| June 20 | 0.49 | July 13 | 0.01 | Sept. 5 | 0.07 | Oct. 1 | 0.01 |
| 21 | 1.00 | 14 | .05 | 12 | .76 | 3 | .40 |
| 22 | .02 | 15 | .02 | 13 | .21 | 4 | .10 |
| 26 | .35 | 20 | .17 | 14 | .01 | 5 | .13 |
| 29 | .11 | 31 | .01 | 15 | .57 | 6 | .38 |
| Total | 1.97 | Total | 1.28 | 18 | .09 | 7 | .22 |
| | | | | 20 | .91 | 10 | .50 |
| July 1 | .02 | Aug. 3 | .09 | 21 | .68 | 11 | .10 |
| 2 | .11 | 23 | .02 | 27 | .09 | 12 | .02 |
| 5 | .03 | | | | | 13 | .07 |
| 10 | .51 | Total | .11 | Total | 3.39 | Total | 1.93 |
| 11 | .35 | | | | | | |
| | | | | | | Total for season | 8.68 |

The beets were grown according to ordinary farm practice, except in the matter of irrigation. They were put in late, however, and a very poor stand was obtained, which accounts for the low yield. Two plots

were irrigated for the purpose of keeping the soil fairly moist at all times. To accomplish this, water was applied three times: July 3, August 10, and August 25. Cultivations were given as follows: June 17, June 30, July 8, and July 20. Two alternate plots were allowed to become quite dry and were irrigated only once during the summer: August 10. They were cultivated on June 17 and July 22. On October 18 each beet was examined for root lice, as in the Huntley experiment.

The combined results from the four plots are given in Table VI.

TABLE VI.—*Record of sugar-beet root-louse increase under different soil-moisture conditions. Bozeman irrigation experiment*

| Number of plots and number of irrigations. | Condition of sugar beets at harvest. | | | | | | |
|---|---|---|---|---|---|---|---|
| | Number of plants uninfested. | Number of plants slightly infested. | Number of plants badly infested. | Number of plants very badly infested. | Percentage of infestation. | Percentage injuriously infested. | Total yield (in pounds). |
| 2 plots, 1 irrigation | 3,304 | 3,124 | 0 | 0 | 48.6 | 0 | 6,[illegible]8 |
| 2 plots, 3 irrigations | 8,612 | 830 | 0 | 0 | 8.8 | 0 | 8,68[illegible] |

Not a beet in any of the plots was injuriously infested at the time of harvest. It is hard to understand why the infestation in all plots was so slight, unless we take into consideration the factor of rainfall. Frequent rains during September and early October kept all the plots quite wet and may have resulted in killing many of the lice.

The experiment, however, showed a marked difference in the number of slightly infested plants on the wet and the dry plots. In the plots irrigated only once 48.6 per cent of the plants were infested, while in the plots irrigated three times the infestation was reduced to 8.8 per cent.

### IRRIGATION EXPERIMENT AT EDGAR

Four plots, each containing 0.45 acre, were used. They were located on the Billings Sugar Co.'s experimental farm and were in charge of Mr. Hans Mendelson, whose friendly cooperation made the experiment at this place possible.

The plots were bordered by a grove of cottonwoods, in which many of the trees bore thousands of galls of the sugar-beet root louse, and during the summer migration period the winged lice could be seen flying in large numbers from the trees to the beets. Upon some plants as many as 8 migrants were seen at one time. Each migrant on an average produces 10 young, which descend to the beet roots; and, when it is stated that in insectary experiments 10 lice have been known to increase to 2,150 in two months, it can readily be understood why the infestation was so severe.

The rainfall from June 15 to September 30 is given in Table VII.

TABLE VII.—*Record of rainfall at Edgar, Mont., from June 15 to Sept. 30, 1914*

| Day. | Precipitation. | Day. | Precipitation. | Day. | Precipitation. | Day. | Precipitation. |
|---|---|---|---|---|---|---|---|
| | *Inches.* | | *Inch.* | | *Inch.* | | *Inch.* |
| June 20.... | 0.30 | July 10.... | 0.40 | Aug. 16.... | 0.20 | Sept. 15.... | 0.15 |
| 26.... | 0.85 | | | 19.... | .11 | 20.... | .80 |
| 29.... | .26 | Total | .40 | | | | |
| | | | | Total | .33 | Total | .95 |
| Total | 1.41 | | | | | Total for season ... | 3.09 |

The sugar beets at Edgar were not thinned, as small beets to be used for seed growing the following year were desired. This accounts for the large number of beets reported upon, over 61,000 in this one experiment. Two plots were so irrigated that the soil was kept fairly moist for the greater part of the growing season. Water was applied June 23, July 9, August 10, and September 2. Cultivations were given as follows: May 30, June 9, July 13, and July 27. Two alternate plots were allowed to become quite dry between irrigations, but they suffered no more from lack of moisture than do many sugar beets under ordinary farm practice. They were irrigated on June 9 and August 10. Cultivations were given on May 30, June 9, and July 13.

Beginning on October 2 each beet was examined for root lice, as in the Huntley and Bozeman experiments. Sugar analyses were made at Edgar by Mr. J. F. Jarrel.

The combined results from all plats are given in Table VIII.

TABLE VIII.—*Record of sugar-beet root-louse increase under different soil-moisture conditions. Edgar irrigation experiment*

| Number of plots and number of irrigations. | Condition of sugar beets at harvest. | | | | | | Average sugar content (per cent.) | Total yield (in pounds). |
|---|---|---|---|---|---|---|---|---|
| | Number of plants uninfested. | Number of plants slightly infested. | Number of plants badly infested. | Number of plants very badly infested. | Percentage of infestation. | Percentage injuriously infested. | | |
| 2 plots, 2 irrigations..... | 1,265 | 19,606 | 5,493 | 3,565 | 95.7 | 30.2 | 16.1 | 11,672 |
| 2 plots, 4 irrigations..... | 9,896 | 18,186 | 2,440 | 700 | 68.0 | 10.0 | 16.5 | 13,169 |

At the time of harvest the percentage of infestation was very high in all plots. In the plots irrigated only twice 95.7 per cent were infested, 30 per cent being injuriously infested. In the plots irrigated four times the total infestation was reduced to 68 per cent, and only 10 per cent were injuriously infested. The wet plots show a slightly better yield in sugar and a decidedly better yield in weight.

Several points of interest which do not show in Table VIII were especially noticeable at Edgar. There, as at Huntley and Bozeman, the irrigation water was not always distributed evenly over the plots, and some of the higher spots generally remained fairly dry, even in the plots that were supposed to be the wettest. When the beets were pulled, such areas were noticeably the most heavily infested. Had it been possible to soak thoroughly all of the surface soil in the wet plots at each irrigation, the infestation in such plots would have been in all probability still further reduced.

One of the wet plots was adjacent to an irrigating ditch which carried more or less water all summer. Moisture seeped from the ditch into the beet field, and at the time the beets were pulled the soil in the first and second rows from the ditch was noticeably more moist than in the remainder of the plot. It is highly significant that each of these rows contained more plants entirely free from root lice than did any of the other 78 rows included in the Edgar experiment. The infestation in the first six rows from the ditch is given in Table IX.

TABLE IX.—*Relative root-louse infestation in six rows of sugar beets close to an irrigating ditch at Edgar, Mont.*

| No. of row from ditch. | Condition of sugar beets at harvest. | | | |
|---|---|---|---|---|
| | Number of plants uninfested. | Number of plants slightly infested. | Number of plants badly infested. | Number of plants very badly infested. |
| 1 | 552 | 393 | 24 | 2 |
| 2 | 496 | 394 | 32 | 7 |
| 3 | 223 | 525 | 76 | 18 |
| 4 | 244 | 456 | 93 | 14 |
| 5 | 114 | 554 | 102 | 21 |
| 6 | 233 | 470 | 116 | 11 |

## SUMMARY OF FIELD EXPERIMENTS

In summing up the results of the irrigation experiments at Huntley, Bozeman, and Edgar, it may be said that sugar beets grown under rather moist conditions were the least infested with root lice and yielded the highest both in sugar content and in tonnage. By combining the results from the three experiments Table X has been constructed.

TABLE X.—*Combined records of sugar-beet root-louse increase under different soil-moisture conditions. Irrigation experiments at Huntley, Bozeman, and Edgar, Mont.*

| Moisture condition. | Number of beets grown. | Condition of sugar beets at harvest. | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Number of plants uninfested. | Number of plants slightly infested. | Number of plants badly infested. | Number of plants very badly infested. | Percentage of infestation. | Percentage injuriously infested. | Average sugar content (per cent.) | Total yield (in pounds). |
| Fairly dry | 43,901 | 7,226 | 27,073 | 5,959 | 3,652 | 83.5 | 21.9 | 15.2 | 25,587 |
| Fairly moist | 48,342 | 23,704 | 21,069 | 2,655 | 784 | 50.7 | 7.1 | 16.4 | 30,833 |

## RELATION OF SOIL MOISTURE TO SUGAR-BEET ROOT-LOUSE CONTROL

From the results obtained by field observation, insectary experiments, and irrigation tests it seems safe to assume that soil moisture is a very important factor in controlling the rate of increase in colonies of the sugar-beet root louse. While root lice were in no instance entirely controlled by irrigation during the first year's experiments, their number was greatly reduced, and it is hoped that a system of irrigation may be worked out that will reduce root-louse injury to a negligible amount. It is expected that such a system will not interfere with the approved sugar-beet cultural methods, but that in the light of experiments reported in this paper it will rather tend to increase both the sugar content and the tonnage, and will thus perhaps more than pay for the extra labor it may demand. The principal immediate source of root-louse infestation of sugar beets is the cottonwood (*Populus balsamifera* L. and *P. angustifolia* James), upon which the root louse develops galls in the spring. During the latter part of June and early July some of the numerous migrants that have developed within the galls fly to sugar beets, where they deposit living young, which descend to the roots and start new colonies. In studying the life history of the sugar-beet root louse in the insectary it has been found very difficult to induce the progeny of the migrants to colonize upon sugar beets growing in soil the surface of which is at all moist. The only successful attempts in colonization have been where sugar beets were subirrigated and several inches of dry soil were kept at the surface. It therefore seems highly important that sugar-beet fields should not be allowed to dry out during the period when the sugar-beet root louse is migrating from the cottonwoods to the beet fields. All too frequently this is just the time when in ordinary farm practice the beet fields are allowed to become quite dry. The June rains cease, and by July 1 in an average year nearly all the beet fields in the Yellowstone Valley need water. Many times water is purposely withheld at this time

because of the mistaken idea that if the fields are allowed to dry out the sugar-beet plant will send its taproot deeper into the soil in search of moisture and thus produce a better-shaped beet. Extremely dry conditions at this time not only check the growth of the plant but offer ideal conditions for the starting of root-louse colonies. The ground is generally cracked open about the base of the plant and the young lice deposited upon the leaves by the migrants have no difficulty in establishing themselves upon the fiber rootlets in the comparatively dry soil, where all conditions are favorable for rapid growth and multiplication. Fortunately the best authorities on sugar-beet culture are urging against allowing the beet fields to become dry at this time, not primarily because of the possibility of root-louse infestation, but because of the general welfare of the crop. Mr. Hans Mendelson, scientist for the Billings Sugar Co., has stated to the writer several times that all of that company's irrigation experiments have shown that early and frequent irrigations produce the highest sugar content and tonnage. In a recent letter he states, "All our experiments in early irrigation have shown that irrespective of aphis it is the right treatment." Thus, by irrigating early, before the fields become dry, the chances for root-louse infestation are reduced and the best conditions for plant growth are secured.

It is also important that plenty of soil moisture be maintained throughout the growing season. Some of the myriads of young lice produced by the migrants from the cottonwoods are almost sure to become established even in fields that are well irrigated. The presence of sufficient soil moisture will tend to retard their increase and by promoting a vigorous plant growth will enable the sugar beets to withstand better the root-louse attacks. A scarcity of soil moisture results in a rapid multiplication of the few root lice that may be in the soil, and plants suffering for lack of moisture are in very poor condition to withstand the drain of root-louse attacks.

The details of this system, such as the number of irrigations per season, the quantity of water to be applied at one irrigation, and the question of cultivation, have not been thoroughly worked out, and the problem will demand several seasons' work before definite recommendations can be made. However, the principle is believed to be sound, and at present irrigation apparently offers the most effective and the most practical method of controlling the sugar-beet root louse.

# A NEW LEAF AND TWIG DISEASE OF PICEA ENGELMANNI

[A PRELIMINARY REPORT]

By JAMES R. WEIR,
*Forest Pathologist, Investigations in Forest Pathology,*
*Bureau of Plant Industry*

The doubt expressed by some mycologists that *Herpotrichia nigra* Hartig and *Neopeckia coulteri* (Pk.) Sacc. are distinct species should be entirely dispelled by the recent article by Sturgis.[1]

These species are chiefly distinguished by the color and form of their spores. The spores of *N. coulteri* when mature are blunt-elliptical, dark-brown, 1-septate, conspicuously constricted at the septa, measuring from material at hand 19.8 to 28.9 by 9.5 to 10$\mu$ (Pl. XXXIV, fig. 1, *A*). Those of *H. nigra* when mature are elliptical, "pale to darker olivaceous-brown," 3-septate. They are not so dark in color, nor are they so conspicuously constricted at the septa as those of the former species. These spores measure from material at hand 21.8 to 31.8 by 7.2 to 9.0$\mu$ (Pl. XXXIV, fig. 1, *B*).

The microscopical characters of the spores of these species are seen to be quite distinct. Throughout the examination of a large amount of material from all parts of the Northwest and from many hosts these characters were found to be constant. It is practically impossible to distinguish the two species by their gross appearance in nature. The mycelium of both involves the leaves and stems of their hosts in a felt-like mass, resulting in the death of the parts infected. Occasionally the mycelium of *N. coulteri* has a lighter color than that of *H. nigra* when in mass, but either species may vary in this respect with age and long exposure. As a rule, the species may be readily recognized by their choice of hosts. When not in contact or intermingled with others, the host plants selected by *H. nigra* invariably belong to some species of the genera Abies, Juniperus, Picea, Libocedrus, or Tsuga.[2] The writer has collected specimens on the above genera and also on Thuya and Taxus. Hartig[3] reports *H. nigra* on *Pinus montana* in Europe. With the exception of very special instances *N. coulteri* is always found associated with the genus Pinus. Occasionally both

[1] Sturgis, W. C. Herpotrichia and Neopeckia on conifers. *In* Phytopathology, v. 3, no. 3, p. 152–158, pl. 12–13. 1913.

[2] Hedgcock, G. G. Notes on some diseases of trees in our national forests. *In* Phytopathology, v. 4, no. 3, p. 183–188. 1914.

[3] Hartig, Robert. Trichosphaeria parasitica und Herpotrichia nigra. *In* Hedwigia, Bd. 27, Heft 1, p. 12–15. 1888.

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these fungi spread to the leaves of an adjacent tree which normally is never selected as a host. This occurs when the branches and leaves of the regular host are intermingled or in contact with those of the other. The mycelium in such cases may be simply crowded over and may continue to draw its nourishment from its regular host. Instances of this kind have been noted where *H. nigra* spread from *Abies lasiocarpa* to *Phyllodoce empetriformis* (Smith) D. Don. Whether the mycelium actually penetrates the tissues of the leaves of the borrowed host has not been determined. The epiphytism of the fungus, however, is sufficient to cause the death of the leaves covered by its mycelium.

So selective are these two interesting fungi with regard to their hosts, it has always been a field practice of the writer to refer them to their respective genera by this fact alone. On the examination of several collections of what seemed to be *H. nigra* on *Picea engelmanni* from Marble Mountain, St. Joe National Forest, Idaho, it was found that the fungus was not this species but another of the same genus. This led to a further examination of the same material and also of other collections from various regions of the West which were sent in from the field labeled "*Herpotrichia nigra*." The fungus on a large part of these collections on species of Picea was found to be quite different in its microscopic characters from *H. nigra*. Although the gross appearance of the mycelial mat was the same (Pl. XXXIV, fig. 2), the mature spores were uniformly 5-septate, and were scarcely constricted at the septa, which were prominent and fairly thick (Pl. XXXIV, fig. 1, *C*). A large amount of material was examined, and the 5-septate spore was found to be as characteristic a feature of this fungus as is the 1-septate spore for *N. coulteri* and the 3-septate spore for *H. nigra*. Mature apothecia of all three fungi were crushed together and mounted on the same slide. Plate XXXIV, figure 1, *A*, *B*, and *C*, are reproduced from camera-lucida drawings of this material and show the proportionate size and character of the asci and spores for all three species.

Since the fungus originally collected on *Picea engelmanni* from Marble Mountain, Idaho, does not agree with *H. nigra* Hartig or with any other known species, it is described as new:

**Herpotrichia quinqueseptata**, n. sp.

Perithecia gregarious or scattered, spherical, 0.19 to 0.43 mm. in diameter, partially embedded in a dark-brown subiculum 0.15 to 0.48 mm. thick, more often free, ostiola not prominent. Asci cylindrical or slightly fusiform, 99.8 to 137.6 by 14.1 to 16.5$\mu$. Paraphyses filiform, fugacious. Ascospores irregularly biseriate in the ascus, fusoid or long elliptical, sometimes slightly curved; when mature, 5-septate, may be slightly constricted at the septa, light brown, 28.3 to 33.8 by 7.6 to 9.05$\mu$.

Type locality.—Marble Mountain, St. Joe National Forest, Idaho.

Habitat.—Living twigs and leaves of *Picea engelmanni*.

Type material has been deposited in the Office of Investigations in Forest Pathology and in the Pathological Collections, Bureau of Plant Industry, Washington, D. C.

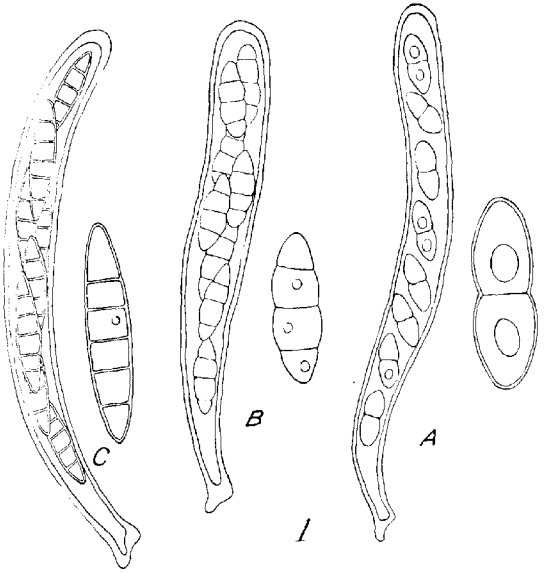
All three of the foregoing species are truly alpine in habit and are not usually found growing normally below an elevation of 5,000 or 6,000 feet. This habit, of course, varies with the latitude. Moreover, they are very common, and from the writer's experience they cause considerable damage to alpine forests, particularly to the younger trees. Young seedlings 4 to 8 years old have been found infected, and old trees frequently succumb to their ravages. In some regions of the Northwest on northern exposures, entire stands when composed mainly of even-aged alpine fir are frequently so generally infected by *H. nigra* as to appear ragged and bare. A sample acre taken on a north slope of Mt. Casey (Selkirks, northern Idaho) at an elevation of 6,735 feet showed 95 per cent of the alpine firs to be infected by this fungus.

The influence of these fungi on their hosts is likewise discernible in the gradual falling off of the annual increment. This causes a sharp contrast in the radial dimensions of the annual rings, even in the finely layered condition of the wood of alpine trees. The dense mat of brown or black mycelium (Pl. XXXIV, fig. 2), often of sufficient thickness and extent to completely spread over entire twigs, burying the leaves entirely in its mass, is enabled to bring about certain phenomena of a very unusual nature. It has been found by actual experiment that this mycelial mat influences the temperature of the enveloped leaves in the same manner as any dark covering acts on the bulb of an air thermometer. The fungus acting as a pronounced epiphyte may thus be enabled through a slight rise in temperature to incubate its own mycelia within the tissues of the leaf and hence may hasten its parasitic activities. Probably the spread of the epiphytic mycelium to leaves not infected internally at the time, as sometimes occurs, is accompanied by reactions of a physiological nature that are highly injurious and pave the way for the advance of the mycelium into the tissues of the leaf. The spread of the mycelium over young growth, from the time the snow disappears in the spring to early fall, is fairly rapid. Branches of alpine fir 2 feet in length that were infected at their tips in the early spring have had their entire leaf surface destroyed by October of the following year.

## PLATE XXXIV

Fig. 1.—*A*, *Neopeckia coulteri*, ascus with mature spores; *B*, *Herpotrichia nigra*, ascus with mature spores; *C*, *Herpotrichia quinqueseptata*, ascus with mature spores.

Fig. 2.—Branch of *Picea engelmanni* infected with *Herpotrichia quinqueseptata*.

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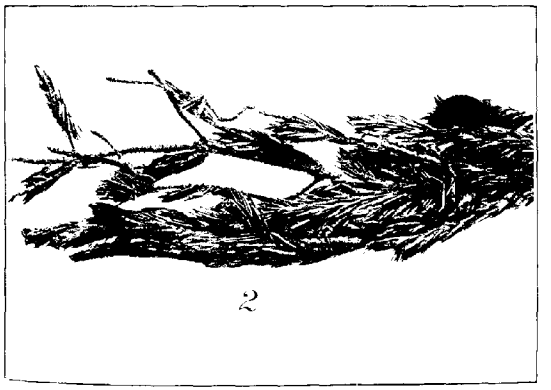

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# SOME SUGAR-CANE ROOT-BORING WEEVILS OF THE WEST INDIES

By W. DWIGHT PIERCE,

*Entomological Assistant, Investigations of Insects Affecting Southern Field Crops, Bureau of Entomology*

## INTRODUCTION

Sugar-cane (*Saccharum officinarum*) growers throughout the world find among their most serious enemies the weevils which bore in the subterranean portions of the stalks and the root crown. The methods of cultivation and propagation in vogue in the sugar-cane industry make an attack of this nature more serious than injury to another part of the plant.

In the West Indies one of the most important groups of economic weevils is the genus Diaprepes, which is mainly confined to the periphery of the Caribbean Sea. The present paper deals with the weevils of this genus which attack sugar cane and which are popularly known as sugar-cane root borers. The writer's purpose is to straighten out the difficult nomenclature, to point out the dangerous nature of the injury by the species treated, and so to describe the various forms that quarantine agents may readily detect them.

These weevils are so variable in color, shape, and markings that it is extremely difficult to come to a definite decision as to their specific limitations. Many names have been used which can only be considered of varietal significance or as mere synonyms. The same species is likely to be known by different names in different islands. It is therefore necessary to go a little deeply into the description of the various forms and to trace the blending from one form to another through the islands. For this reason the technical matter must receive more attention than the biological and economic data belonging with it.

## GENUS DIAPREPES

### THE ADULT

The genus Diaprepes Schönherr contains many species found in the West Indies, Mexico, and Central and South America. It is typically tanymecine in the possession of distinct ocular vibrissæ on the prothorax in the majority of species, although occasional specimens of undoubted Diaprepes are found without trace of the vibrissæ. The genus is distinguished by the peculiar irregularities of the elytral striæ, which num-

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ber over ten. The species attacking sugar cane belong to the group having three rostral carinæ and are to be separated as follows:

*Classification of sugar-cane species and varieties of Diaprepes*

I. Beak with three rostral carinæ but not otherwise rugose; first funicular joint very much smaller than second; densely squamose, more or less denuded in vittæ. Prothorax not conspicuously transverse, usually a little longer than wide; vibrissæ distinct; body with a lateral yellow or pink vitta.....*spengleri* Linnaeus.

1a. Median rostral carina with fovea near apex; elytra without denuded intervals; undersides evenly pubescent; pubescence consisting principally of flat scales.....variety *marginatus* Olivier.

1b. Median rostral carina without fovea but with transverse carina at same point connecting with the lateral carinæ; elytra with denuded intervals; pubescence throughout consisting of flat and upright scales.

2a. Elytra with only a single row of punctures between the two humeral denuded intervals.

3a. Fifth interval denuded at base.....variety *comma* Boheman.

3b. Fifth interval denuded at base, and third on disk.
variety *spengleri* Linnaeus.

3c. Third and fifth intervals denuded at base. variety *abbreviatus* Olivier.

3d. Third to tenth intervals denuded....variety *denudatus*, new variety.

2b. Elytra with two rows of punctures between the two humeral denuded intervals; third and fifth intervals denuded at base.
variety *festivus* Fabricius.

II. Beak with three rostral carinæ and closely recticulately rugose; first funicular joint very nearly as long as second; sparsely squamose.....*famelicus* Olivier.

DIAPREPES SPENGLERI LINNAEUS

No species hitherto studied by the writer has shown such a wide range of variations in colors and markings as *Diaprepes spengleri* Linnaeus. The two sexes differ so much in shape that the male of one variety and the female of another would easily pass as very distinct species. The structural and sculptural differences, however, are exceedingly minute, and the large series of specimens from Porto Rico, Barbados, and the intermediate islands shows that they must all be one species.

The following characters are common to all forms of the species and are therefore of specific weight in comparing this species with others in the genus:

Beak tricarinate, with indications of a transverse carina near apex; nasal plate emarginate, triangular. Antennal scrobes arcuate, passing immediately beneath the eye; scape elongate, clavate; funicle with second joint longer than first, third joint a little longer than the following, which are slightly longer than wide and subequal; club elongate, pointed, and very finely pubescent. Prothorax very irregularly confluently punctured, sparsely pubescent above, laterally densely vittate; truncate at apex, with distinct ocular vibrissæ. Scutellum subquadrate, rounded behind. Elytral striæ confused from sixth to sides. Elytra of female acute and somewhat sinuate on margin behind; elytra of male less acute, with margin convex. Last ventral segment of female triangular, of male broadly rounded behind. Tibiæ with a few small denticles.

THE IMMATURE STAGES

A sketch of an egg is given in Plate XXXVIII, figure 1, *F*.

The larvæ of weevils are most readily distinguished one from another by the folds of the body, the shape and arrangement of the spiracles, the head, and the caudal segment. Sketches of these parts are therefore given to assist in the identification of larvæ of *Diaprepes spengleri*. These drawings have been made with great accuracy with the intention of bringing out the systematic characters (Pl. XXXVII, fig. 2, and Pl. XXXVIII, fig. 1, A–E).

In the pupæ of weevils we find even better characters for separating species. The general form of the pupa and the arrangement of the ventral parts is of considerable importance (Pl. XXXVIII, fig. 2). The mouth parts of this species are very interesting and have been illustrated in Plate XXXVIII, figure 2, *B*. The arrangement of the tubercles on the dorsal segments is important, especially in the scutellar region (Pl. XXXVIII, fig. 2, *C*), and on the apical segments (Pl. XXXVIII, fig. 2, *E*). But more important than any other characters are the structures of the last three or four ventral segments (Pl. XXXVIII, fig. 2, *D*). It is hardly necessary to add much of a descriptive nature to the figures given. The spiracles of the abdomen are dark and very plain on the first to fifth segments, and very inconspicuous on the sixth to eighth segments. The thoracic spiracle is elongate and located between the prothorax and mesothorax.

VARIETIES OF DIAPREPES SPENGLERI

Merely for the convenience of designation and to retain old, well-known names the species *Diaprepes spengleri* has been arbitrarily arranged into varieties by the writer.

The variety which is presumably nearest the parent variety, *Diaprepes spengleri marginatus* Olivier, is only at hand from St. Croix. The next step in the progression of the species has been called *D. spengleri comma* Boheman, and ranges from the Dominican Republic and Porto Rico to Dominica; in other words occurring in both directions from the home of *D. spengleri marginatus*, with nearest approach to this form in a specimen from Dominica. The next form is merely an intermediate and is found in the collection only from Porto Rico. This form most nearly answers the description of typical *D. spengleri* Linnaeus. The fourth variety, *D. spengleri abbreviatus* Olivier, is at hand from Porto Rico, Montserrat, Dominica, and Barbados. There seem to be two trends of modification from this. The fifth variety, *D. spengleri denudatus*, n. var., is an extreme from a trend found in the Porto Rican material, and is at hand only from Guadeloupe. The sixth variety, *D. spengleri festivus* Fabricius, is from the branch of the fourth found in Dominica and Barbados, and is at hand from Barbados and St. Vincent.

Owing to the remarkable differences displayed in the species, a more or less detailed study follows to show why the writer has considered

these apparently unrelated forms all as one species. Over 40 different variations are at hand. The color names used are according to Ridgway's Color Standards.[1]

Material has been examined belonging to the United States National Museum, the Porto Rican Sugar Growers' Association, the Porto Rico Experiment Station, and the Imperial Agricultural Department of Barbados. Over 250 specimens of this species are now at hand, and, in order to show the trend of variation, the different forms are briefly described.

**Diaprepes spengleri marginatus** Olivier.

*1.* The impressions on the thorax are clad with flat white scales, which also form the general color of the vestiture of the body, except for a pale ocherous lateral vitta on the prothorax and elytra, and a trace of ocherous near the scutellum on the elytra. The elytra are uniformly squamose, with flat scales, without any denuded areas whatever. The material at hand consists of four specimens from St. Croix, two collected by Mr. Longfield Smith on cotton in May, 1912, and two collected by Mr. V. Hanchell on July 31, 1908. Size, 14 to 18 mm. This form is not absolutely linked to the following. Except for the lack of denudations it could not be readily distinguished from *D. spengleri festivus* (Pl. XXXV, fig. 1).

*D. spengleri marginatus* has been recorded by Fleutiaux and Sallé on *Chrysobalanus icaco* in Guadeloupe and was collected by Longfield Smith on cotton.

**Diaprepes spengleri comma** Boheman.

*2a.* The impressions on the thorax are clad with iridescent light blue-green and whitish scales, which also form the general color of the elytra and undersides, except for a broad lateral vitta of empire yellow on the elytra, and a touch of the same on each side of the scutellum. The basal third of the fifth interval, the basal half of the ninth interval, and the basal fourth of the tenth interval are denuded and shining black. One specimen is at hand from Romana, Dominica, collected by Mr. W. V. Tower on April 16, 1913. Size, 14 mm.

*2b.* The tendency in this variety is to increase the amount of the denuded area. The majority of the specimens are less greenish and have the basal half of the fifth interval, from one-half to three-fourths of the ninth, and from one-fourth to one-half of the tenth denuded. The lateral yellow vitta is distinct, but the yellow sutural spots are sometimes lacking. The material consists of one specimen from Dominica, collected by Mr. H. W. Foote in June or July (Yale Expedition, 1913); fourteen specimens from the Dominican Republic, collected by August Busck in August; five specimens from Guanica, and one from Santa Isabel, Porto Rico, collected by Messrs. E. G. Smyth and D. L. Van

[1] Ridgway, Robert. Color Standards and Color Nomenclature. 43 p., 53 col. pl. Washington, D. C., 1912.

Dine on May 28, 1913, and October 20, 1910. The size varies from 11 to 15 mm. (Pl. XXXV, fig. 2).

*2c.* Another direction for the variation lies in the color of the scales. Three specimens from Guanica and Santa Isabel, Porto Rico, have the empire-yellow scutellar area and lateral vitta very distinct, but the remainder of the elytra is clad with tawny-ochraceous. These were all collected in May. Size, 11 mm.

*2d.* The yellow sutural spots and lateral vittæ are here replaced by shrimp pink in four specimens, which are almost white in general vestiture, and in two specimens, which are colored tawny-ochraceous, all from Guanica, Porto Rico. One of the latter has the deciduous pieces of the mandibles still intact. Size, 11 to 16 mm.

*2e.* This form completely lacks the colored lateral vitta. A specimen from Humacao, Porto Rico, collected on November 12, 1910, by Mr. D. L. Van Dine, and one from Bayamon, Porto Rico, collected by Mr. A. Busck in January, 1899, have the greenish scales predominant, while specimens from Guanica and Santa Isabel, Porto Rico, have the whitish scales predominant. Size, 9 to 18 mm.

*2f.* The next change consists in the intensification of the green scales to shining pale yellow-green in specimens from Humacao, Barceloneta, and Canovanos.

*2g.* Then follows an admixture of a few ochraceous-tawny scales, especially posteriorly, in a specimen collected on sugar cane at Yabucoa, Porto Rico, by Mr. D. L. Van Dine on April 20, 1911.

*2h.* The next modification is the strong admixture of ochraceous-tawny scales on all parts of the elytra except near the suture, while on the thorax and beneath, all the scales remain green, as found in a specimen from Bayamon, Porto Rico, collected by Mr. Van Dine on October 9, 1910. Size, 11 to 14 mm.

*2i.* In the final gradations of this variety the general scale coloring of the elytra is light buff to ochraceous-tawny, numerous different tones being found in the present series. In several specimens the body color is a dark Hessian brown. Two specimens from Yauco, Porto Rico, collected in May, 1912, and two collected on sugar cane by Mr. J. R. Johnston on June 11, 1911; four specimens from Santa Isabel, collected on sugar cane by Mr. Van Dine on May 30, 1911; a single specimen from Utuado, Porto Rico, in January, 1899, Mr. Busck, collector; two specimens from Barceloneta, P. R., on May 16, 1911, collected by Mr. J. R. Johnston. Size, 12 to 16 mm.

A newly reared specimen from Rio Piedras, collected on February 8, 1912, on sugar cane by Mr. J. R. Johnston, has the mandibles complete. The deciduous pieces are long, shining, curved, overlapping at tips, with edges sharp and the tips acute, with the upper surface strongly convex, and the lower surface concave. These pieces are longer than the first two funicular joints together.

*D. spengleri comma* occurs in the Dominican Republic, Porto Rico, and Dominica. In Porto Rico it intergrades by almost imperceptible changes into the forms here named *D. spengleri spengleri* and *D. spengleri abbreviatus*. On this island it has been taken at all times of the year at Buena Vista, Guanica, Yabucoa, Luquillo, and Santa Rita, by Messrs. D. L. Van Dine, T. H. Jones, E. G. Smyth, J. R. Johnston, and C. T. Murphy, on sugar cane, grass, jobo (*Spondias lutea*), bledo (*Amaranthus* sp.), and *Parthenium* spp., and has been found in all stages at the roots of sugar cane.

**Diaprepes spengleri spengleri** Linnaeus.

*3*. The third variety also lacks a different-colored lateral vitta, but has a short median denuded area on the third interval. This line varies from a dot to several millimeters in length, but is usually distant from the base. I take this variety to be typical *D. spengleri spengleri*. Size, 8 to 16 mm. (See Pl. XXXV, fig. 3.)

*3a*. The first form, with white scales only, is represented by a specimen from Yabucoa, taken on sugar cane on April 20, 1911, by Mr. D. L. Van Dine.

*3b*. Three specimens from the same place have the scales light yellow-green. Five in various tones of green come from Yauco and Salinas, Porto Rico, collected during May.

*3c*. A specimen taken on sugar cane at Fajardo on January 25, 1911, by Mr. D. L. Van Dine is light yellow-green with an admixture of tawny-ochraceous scales.

*3d*. By far the majority of this variety are of light buff to ochraceous-tawny in many different tones. These specimens come from Santa Isabel, Guanica, Cidea, Bayamon, Ponce, Rio Piedras, San Juan, and Maunabo, Porto Rico. One specimen from Cidea was taken by Mr. F. D. Gardner as it was injuring the orange.

*3e*. Two specimens from Rio Piedras of the ochraceous-tawny color were apparently collected in copulation on January 11, 1912. The female has the last ventral segment almost in the form of an isosceles triangle, with the apex narrowly rounded. The male anal segment is transverse subtriangular, with the apex broadly rounded and the surface very rugose. Two other pairs agree in these characters.

*3f*. This form is merely an intermediate between *D. spengleri spengleri* and the next variety. It ranges in color exactly as the preceding, but has elytral intervals 1 to 5 or 6 more or less broadly denuded. The material is from Aguadilla, Utuado, Bayamon, Fajardo, and Mayaguez in the Busck collection, and from Humacao, Guanica, Yabucoa, Casovanas, Rio Piedras, and Ponce in Mr. Van Dine's collection, most of which was taken on sugar cane. One specimen, collected at Cidea by F. D. Gardner, was injuring the orange. Size, 9 to 16 mm.

*D. spengleri spengleri* Linnaeus occurs only in Porto Rico. This form intergrades perfectly into both *D. spengleri comma* and *D. spengleri abbre-*

*viatus* in such a way as to leave no doubt that the three are one species, although the extremes appear so different. It has been taken throughout the year at Buena Vista, Guanica, Santurce, Rio Piedras, Fajardo, Yabucoa, Luquillo, Arecibo, and Cidea, by Messrs. Van Dine, Tower, Jones, Smyth, Johnston, Murphy, and Gardner on sugar cane, grass, *Mimosa* spp., *Ceratonia* spp., guava (*Psidium guajava*), avocado (*Persea gratissima*), mango (*Mangifera indica*), rose, *Spondias lutea*, *Amaranthus* spp., *Parthenium* spp., and orange (*Citrus aurantiaca*). It has been reared from the roots of the orange and sugar cane.

Eggs were obtained and described in September, 1912, by Mr. Jones. They are oblong, oval, smooth, glistening, milky white, with a rather tough membrane, and measure about 1.2 by 0.4 mm. when newly laid. In confinement the females laid the eggs between the surfaces of two leaves, the leaves being brought together and their surfaces about the egg held by an adhesive substance placed between the eggs and around the cluster. The eggs are placed in no regular pattern and are so closely pressed together that their shape is altered. When first laid, the eggs are of a uniform milky white, but within a day after being deposited clear spaces appear at both ends, being more pronounced at one end. Before hatching, these clear spaces disappear, the egg takes on a faint brownish tinge, and the mouth parts of the larva can be seen through the membrane.

The newly emerged larvæ are white, with a slight brownish tinge, have light-brown heads, and are a trifle more than 1 mm. in length. They immediately enter the ground and begin feeding on the root system of the sugar cane or other host.

**Diaprepes spengleri abbreviatus** Olivier.

*4a.* The fourth variety varies in scale color from white through green to ochraceous and sometimes has a yellow spot at the sides of the scutellum, as in the preceding varieties. It also has the lateral vitta of empire-yellow, except in the whitest specimens. The denudations of the third and fifth elytral intervals are almost equal and the intervening intervals are very narrow. The undersides are very sparsely squamose. Mr. Van Dine's material is from Anasco, Guanica, Ponce, Arecibo, and Barceloneta. Five specimens are at hand from La Yoslina, Porto Rico, collected on July 30, 1900, on shade trees. One is labeled "*Diaprepes abbreviatus.*" Some specimens show a small denuded postmedian line on the seventh interval. Size, 10 to 13 mm. (Pl. XXXVI, fig. 1.)

*4b.* This form is light-buff colored in its vestiture and lacks the lateral yellow vitta. The third and fifth intervals are more widely separated. One specimen with black integument from Guanica, Porto Rico, and six specimens with Hessian-brown integument from the Island of Dominica were collected by A. H. Verrill. Size, 9 to 15 mm.

*4c.* The next variation has the denuded intervals more or less widely separated. It is denuded as in the preceding, but also has the ninth interval denuded almost to the apex. The vestiture is in various tones of bluish green with an ocherous area on each side of the scutellum and an ochraceous-tawny lateral vitta. Three specimens are from the island of Montserrat, collected on March 1 and April 2 and 9 by Mr. H. G. Hubbard. One specimen is labeled "*Diaprepes abbreviatus.*" There are also four whitish specimens from Arecibo and Barceloneta, Porto Rico. Size, 14 to 16 mm.

*4d.* The next form is similarly denuded except that the denuded space on the ninth interval is united to that on the eleventh at about the middle of the elytra. The vestiture is intermixed white and golden green. Two specimens from Barbados were collected by Mr. J. Morris, on May 22, 1900, and fourteen were collected by Mr. H. A. Ballou. These are all males of the form *Diaprepes spengleri festivus.*

*D. spengleri abbreviatus* occurs in Porto Rico, Dominica, Montserrat, and Barbados, and occurs on sugar cane in both Porto Rico and Barbados. The Porto Rican material assigned to this form does not closely resemble that from Barbados, but the variation in the Porto Rican material from the *D. spengleri* type is so great that at the other extreme specimens identical with those from Dominica and Montserrat can be found. In the same way the Montserrat material varies to the typical Barbados material, which incidentally is all male. In Guadeloupe it has been found on avocado, coffee, and *Cajanus indicus.*

**Diaprepes spengleri festivus** Fabricius.

*5a.* The fifth variety differs from the preceding by having a double row of punctures between the two humeral denuded intervals. It has an ocherous lateral vitta and spots on each side of the scutellum. The discal denudation of the seventh interval is highly variable, sometimes being connected in front to the ninth interval at about the middle of the elytra. The vestiture of some specimens is white and of the others greenish. Twenty-one specimens, probably *D. spengleri festivus* Fabricius, from Barbados, mostly females, were collected by Mr. H. A. Ballou (Pl. XXXVII, fig. 1).

*5b.* The next form is like the preceding, but without any denudations of the ninth interval and without ochraceous markings. One specimen from St. Vincent was collected by Mr. H. H. Smith and labeled *D. spengleri* by Mr. Champion.

*5c.* The next variation is like the preceding, but has the denuded portion of the third interval short, discal, and the vestiture uniformly buff-yellow. Four specimens from St. Vincent were collected by Mr. H. H. Smith, one of them on the castor plant (*Ricinus communis*). They are labeled *D. spengleri* by Mr. Smith.

*D. spengleri festivus* differs only by the humeral strial punctuation from the preceding. This form is found in Barbados and St. Vincent. It

breeds in the roots of sugar cane, Indian corn (*Zea mays*), Guinea corn (*Andropogon sorghum*), sweet potatoes (*Ipomoea batatas*), Bahama or Bermuda grass (*Capriola dactylon*), and limes (*Citrus medica acida*) in Barbados, and has been collected also on pigeon-pea (*Cajan indicum*) and Bonavist bean (*Dolichos lablab*) in Barbados and on castor plant in St. Vincent. Mr. H. A. Ballou has written considerably on this form under the name "*Diaprepes abbreviatus*." He found the eggs in small clusters on the leaves of a variety of plants. On sugar cane the eggs are usually laid near the tips where the leaves have been split by the wind, the two portions of the leaf being stuck together over the eggs. As many as 89 eggs have been found in a cluster. The young larvæ in attacking sugar cane are first found on the fibrous roots, but as they grow larger they tunnel into the underground stem portions of the plant. Mr. Ballou places the life cycle at about a year, of which about 10 days are spent in the egg stage, 300 in the larval stage, 15 in the pupal stage, and about 20 in the adult.

**Diaprepes spengleri denudatus,** n. var.

6. The opposite extreme from *D. spengleri marginatus* is found in one specimen collected by Mr. H. W. Foote in Guadeloupe in June, 1913. This specimen has the greenish and whitish scales in the dorsal punctures with an ochraceous spot on each side of the scutellum, an ochraceous lateral line on the elytra, and a very white lateral vitta on the prothorax, but the second elytral intervals are the only dorsal intervals clothed with scales. Interval 1 and intervals 3 to 10 are denuded. Size, 12 mm. (Pl. XXXVI, fig. 3.)

DIAPREPES FAMELICUS

The species *Diaprepes famelicus* Olivier differs from *Diaprepes spengleri* in that the three carinæ of the beak are indistinct and the surface is very rugulose. The ocular vibrissæ are almost completely lacking. The species is black and very sparsely inconspicuously squamose. The strial punctures are larger and the striæ closer together than in *D. spengleri* (Pl. XXXVI, fig. 2).

The species is also known as *D. exuriens* Gyllenhal in some of the islands.

Material is at hand from Montserrat, Dominica, and St. Kitts.

*D. famelicus* attacks sugar cane in St. Kitts. Mr. H. G. Hubbard collected several specimens that were notching the leaves of the lime in Montserrat on March 31, 1894.

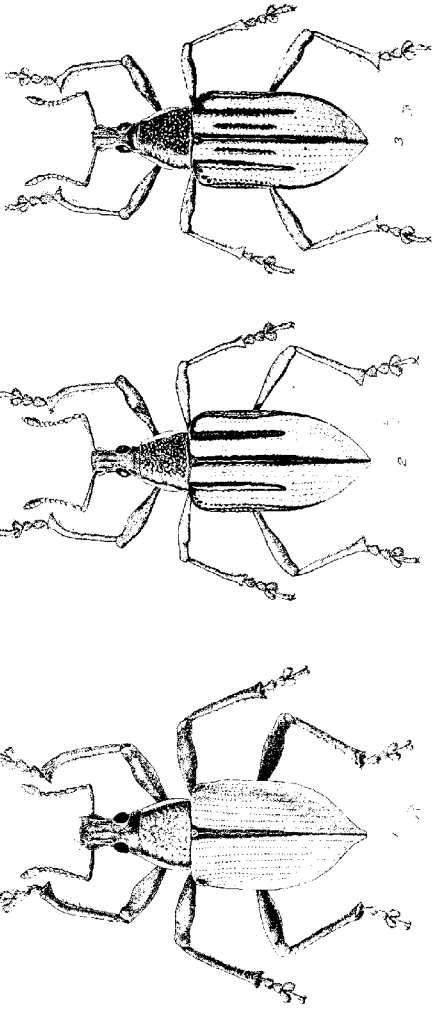
CONTROL OF THE SUGAR-CANE ROOT BORER

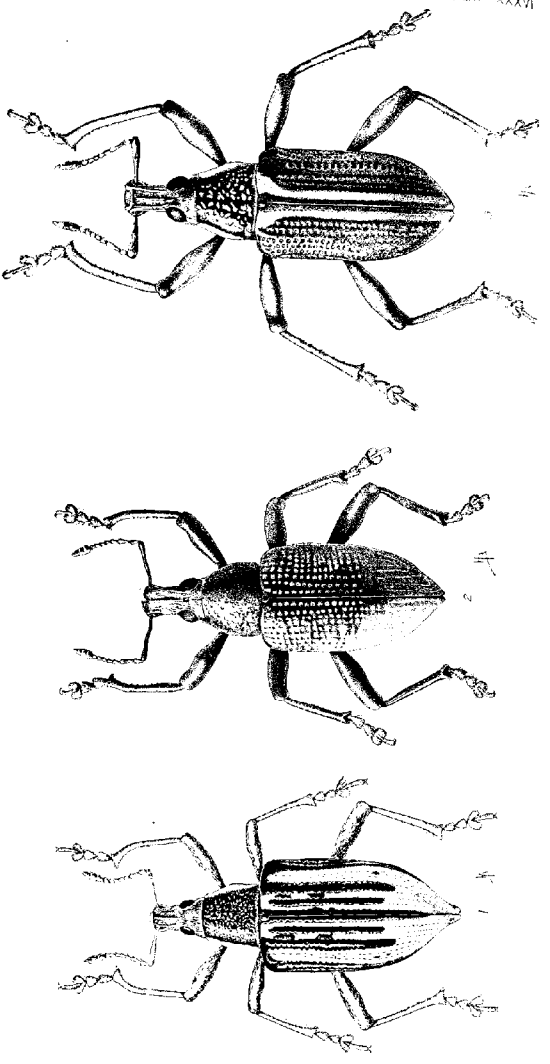
As means of control, Mr. W. V. Tower has suggested spraying with arsenate of lead the trees attacked by adults. Mr. H. A. Ballou suggests rotation of affected crops with unaffected crops, breaking up infested stumps to expose the grubs to the attack of ants and birds, and the subsequent burning of these stumps. He also recommends the hand picking of adults from April to June.

## PLATE XXXV

Varieties of the sugar-cane root borer (*Diaprepes spengleri*)

Fig. 1.—Variety *marginatus*, female from St. Croix.
Fig. 2.—Variety *comma*, male from Porto Rico.
Fig. 3.—Variety *spengleri*, male from Porto Rico.
Drawn by Mr. Harry Bradford.





## PLATE XXXVI

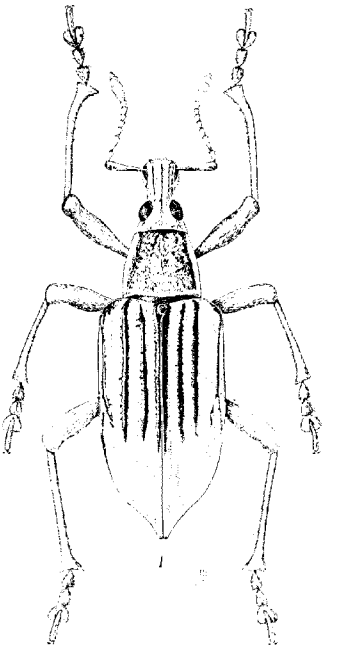


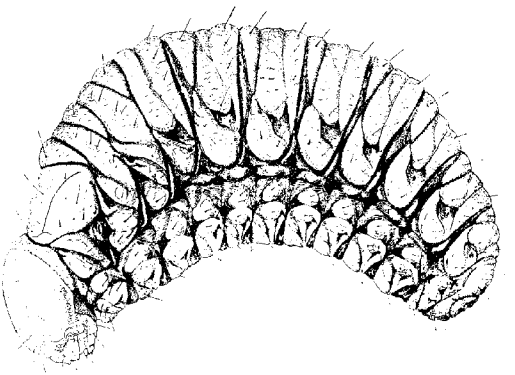
Fig. 1.—*Diaprepes spengleri*, variety *abbreviatus*, female from Porto Rico.
Fig. 2.—*Diaprepes famelicus*, male from St. Kitts.
Fig. 3.—*Diaprepes spengleri denudatus*, new variety, male from Guadeloupe.
Drawn by Mr. Harry Bradford.

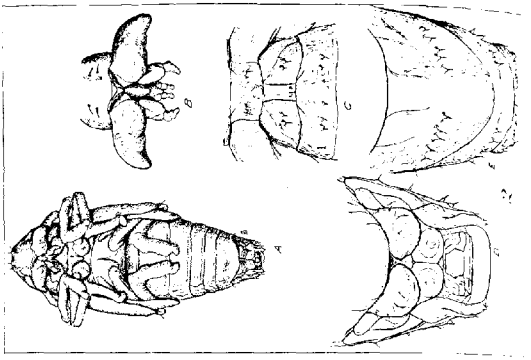
## PLATE XXXVII

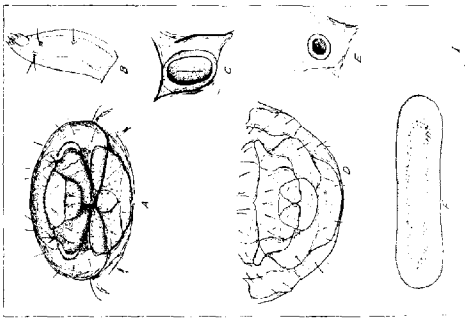
*Diaprepes spengleri*, variety *festivus*

Fig. 1.—Female from Barbados. Drawn by Mr. Harry Bradford.
Fig. 2.—Larva from Barbados.









## PLATE XXXVIII

Fig. 1.—*Diaprepes spengleri*, variety *festivus*: *A*, Face of larva, much enlarged; *B*, maxilla of larva, very greatly enlarged; *C*, thoracic spiracle of larva, very greatly enlarged; *D*, third abdominal spiracle of larva, very greatly enlarged; *E*, last abdominal segments, ventral view, much enlarged; *F*, egg, very greatly enlarged (natural size, 1 mm.). Drawn by the author.

Fig. 2.—*Diaprepes spengleri*, variety *spengleri*: *A*, Pupa, ventral view (natural size, 19 mm.); *B*, mouth parts of pupa, very greatly enlarged; *C*, mesonotum, metanotum, and first abdominal segment, very greatly enlarged; *D*, ventral view of part of the seventh, and the eighth, ninth, and tenth segments, much enlarged; *E*, dorsal view of seventh, eighth, and ninth segments, much enlarged. Drawn by the author.

# A CONTRIBUTION TO THE LIFE HISTORY OF SPONGOSPORA SUBTERRANEA

[A PRELIMINARY REPORT]

By L. O. KUNKEL,

*Pathologist, Office of Cotton- and Truck-Disease Investigations, Bureau of Plant Industry*

## INTRODUCTION

The manner of infection of tubers of the potato (*Solanum tuberosum*) by *Spongospora subterranea* seems never to have been observed. Osborn (14)[1] has described and figured what he considers a single amœba in a potato cell, but as will be seen from the writer's descriptions this can not be regarded as a significant step in the process of infection. It has usually been assumed that each potato cell becomes parasitized by one, or at most only a very few, amœbæ in much the same way as suggested by Woronin (17) for cabbage cells attacked by *Plasmodiophora brassicae*.

*Spongospora subterranea* was first technically described by Wallroth (16) in 1842 as *Erysibe subterranea*. Although it seems to have been widely distributed in Europe, it received little attention until 1885, when Brunchorst (2), a Norwegian botanist, made a careful study of the disease. His observation of plasmodia in the potato cells led him to place it in the group of the Plasmodiophoraceae. It was his opinion that the organism could live saprophytically in the soil, and he states that it is a general belief among farmers that lime and excess moisture favor the disease.

In 1892 Von Lagerheim (9) reported *S. subterranea* as very generally distributed in Quito, South America, which is the native habitat of the potato and may also be the home of Spongospora.

Johnson (7) studied the germination of the spores and describes eight swarm spores coming from each cell of the spore ball. In stained preparations he thought that he was able to see approximately eight nuclei in some of the ungerminated spores. Johnson did not observe the way in which infection takes place, but suggests that the swarm spores find their way into the tuber through lenticels, sprouts, and wounds.

Massee (10) published an account of the disease in 1908. According to his description (11), which is very brief, the spores are uninucleate and on germination produce only one amœba. He did not observe infection, but thinks that the amœbæ may be able to infect new cells by passing through the pits present in the cell wall.

[1] Reference is made by number to "Literature cited," p. 278.

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G—49

Osborn (14) was the first to make an extended cytological study of *S. subterranea* and described for the first time the formation of plasmodia from separate amœbæ within the host cell. He also discusses vegetative nuclear divisions, nuclear fusions, and supposed reduction divisions, but his description, as well as his figures, gives the impression that he has been rather free in interpreting what he observed. Osborn (14) saw the division of infected host cells and gives this as the only method by which the disease spreads in the tissues. He describes a single uninucleate amœba in a young potato cell as the earliest stage with which he is acquainted. This part of his description agrees with observations previously made by Horne (5).

In the early spring of 1913 Güssow (4) reports having received specimens of *S. subterranea* from Quebec and other provinces of Canada. This is the first account of the disease in North America. During 1913 powdery scab was found in one of our chief potato sections by Melhus (12), who reported it on the 1912 crop of northern Maine. This section of Maine joins the Province of New Brunswick, Canada. The presence of Spongospora in the United States raises the question as to the effect this introduced parasite will have on our potato industry in the various parts of the country. This paper deals primarily with certain fundamental facts relating to the life history of the organism and its parasitic relations to the potato, which, as indicated above, are very imperfectly understood. In the author's studies of the germination of the spore balls of *S. subterranea* it has been found that a large number of small plasmodia are produced by the fusion of the amœbæ on certain cultural media. These results, coupled with previous observations in microtome sections of very young sori, show unmistakably that infection takes place through invasion by a plasmodium.

## INFECTION OF YOUNG TUBERS

During the summer of 1913, while stationed at a field laboratory at Caribou, Me., the writer was able to obtain abundant material of *S. subterranea*. Various stages of the disease on young tubers, were fixed in Flemming's solution. This material was embedded in paraffin in the usual way and has been the source of most of the sections from which this study was made. Material for the study of spore germination was obtained from the crops of both 1913 and 1914.

On August 20 some young potato tubers were brought into the laboratory showing brownish-colored blisters easily recognized as very early stages of *S. subterranea*. Others showed none of these blisters, but instead very small, inconspicuous, light-brown-colored circular spots. These spots were never more, and usually less, than ¼ mm. in diameter. On careful observation it could be seen that each of the tiny brownish spots was surrounded by a circular translucent area that varied from 1 to 2 mm. in diameter. In most cases its limits could easily be distinguished. The light, brownish spot at or very near the center of the translucent area was

not sharply limited, but gradually faded out along its edges. On the whole, these spots, each surrounded by a faintly grayish colored, translucent ring, presented much the appearance that might be obtained by injecting a small drop of water beneath the epidermis of a young tuber. A study of free-hand sections through some of these spots led to the conclusion that they were caused by a small, disk-shaped drop of light grayish colored material just beneath the epidermis. Some of these spots were fixed in Flemming's stronger solution and were labeled "halo stage of Spongospora," because when held in the sunlight the small brownish spot had the appearance of being surrounded by a halo. Some of this material was sectioned and stained along with other early stages of the disease before plasmodia had been observed in culture. Saprophytic plasmodia produced in culture media have been studied parallel with investigations of the microtome sections through the translucent areas.

Early and late stages of infection have been obtained, and many of the slides show with great clearness that the potato tissue is first invaded by the fungus in its plasmodium stage. The light-brown spot at the center of the translucent area above described is undoubtedly the point at which the plasmodium enters the skin of the tuber. The limits of the translucent area mark the distance to which the plasmodium has spread beneath the epidermis.

In stained sections it is possible to observe in detail the manner in which the potato tissue is attacked by the plasmodium. Some of the preparations show it passing down through and between the epidermal cells. Usually a considerable number of cells are killed at the point where the plasmodium enters. Once beneath the epidermis, it spreads out in all directions and forms a rather flat, disk-shaped mass, which separates the epidermis from the tissue beneath. In general, the potato cells in contact with the plasmodium are at once stimulated to abnormal growth and division, but some of them are killed as the plasmodium spreads out over the healthy tissue. In this way it comes to occupy a space between the uplifted epidermis and sound tissue beneath. This circular plasmodial mass is thicker at the point where it enters the epidermis than toward the edges. At first the lower surface of the disk-shaped mass is almost smooth. It comes in intimate contact with the cells on which it rests. Soon, however, a number of projections of pseudopodia begin to extend downward, push in between the cells of the sound tissue, and seem to crowd them apart. All of the cell walls that have been or are in contact with the plasmodium stain differently from those in healthy tissue, and such cell walls are often somewhat swollen, showing a special affinity for the orange stain of the triple combination. This indicates that the cellulose is being acted on and in some manner changed, but whether the change is accomplished through the secretion of an enzym the writer is unable to say. It is possible that

such changes might be the result of the direct action of the protoplasm of the plasmodium on the cellulose.

As the pseudopodia push down between the cells the walls of the latter seem to become somewhat gelatinous and the middle lamella is dissolved. These tapering plasmodial projections sometimes extend down into the sound tissue for a distance of five or six cell layers; often, however, they do not go deeper than two cell layers. They are irregular in shape, as might be expected from the way in which they crowd between the cells. Through these pseudopodia the plasmodium comes in contact with a large number of cells. The cell walls seem to become more and more softened and gelatinous as they thicken, and may even lose to a certain extent their original shape, becoming wavy. Plate XXXIX, figure 4, shows the characteristic way in which the plasmodium pushes down between the cells. Deeply stained globular bodies are also conspicuous in it, two of which are sometimes joined together. In certain portions of the plasmodium nuclei can be distinguished, but they are poorly fixed. Plate XL shows a small part of an infecting plasmodium. The cell containing the large nucleus is not yet infected, but the one to the right of this has been penetrated. In Plate XXXIX, figure 3, is shown a vertical section through the parenchyma of the potato and an infecting plasmodium which can be seen spreading out over the healthy tissue and forcing the cells apart. Certain cells have already become infected; many of them are beginning to enlarge. They are stimulated to abnormal growth even before penetration, which suggests that the stimulus may be due to a secretion from the plasmodium.

Through the softened cellulose walls the plasmodium sends small protoplasmic strands, which may for convenience be termed the "infecting pseudopodia." These usually pass into the cell through openings that are quite small, but occasionally the opening is rather large, as shown in Plate XXXIX, figure 5. In this figure is shown a potato cell that is being infected. The plasmodium has made an opening in the wall and is flowing through and apparently into the protoplasm of the cell. The nuclei of *S. subterranea* are well stained. The large nucleoli stain a bright red; the chromatin strands, blue. The host nucleus is also clearly shown. A large nucleolus and chromatin strands can be seen. In some manner which has not yet been determined the infecting pseudopodia become separated from the remainder of the plasmodium, and in this way the individual cells receive each a small portion of protoplasm from the plasmodium that is invading the tissue. The quantity of infecting material received by different cells is quite variable. The writer has not been able to decide what it is that determines the amount received by a given cell. Within the cell the little plasmodium often travels along the cell wall for a certain distance before actually entering the protoplasm of the host. Just how the plasmodium actually penetrates the protoplast of the host cell is a point that is not yet clear.

The stages by which it passes through the limiting membrane and into the protoplasm of the host have not been observed. This is partly due to the fact that most of the cells that are becoming infected or are newly infected are more or less plasmolyzed. Whether this plasmolysis is entirely due to fixation or whether it is in part due to the attack of the plasmodium is an open question. Numerous cases can be found in which the plasmodium has not yet gone far into the host protoplast. In such cases it is usually on that side of the cell from which it entered. Other stages can be found in which the plasmodium has just reached the host nucleus. In a later stage it completely or almost completely surrounds the host nucleus. This may properly be considered the last stage of infection.

The plasmodium is at no time obviously delimited from the protoplasm of the host, and there seem to be no membranes between the two. The meshes in the cytoplasm of the plasmodium are smaller than those in the cytoplasm of the host. The parasite takes the orange stain more intensely than the protoplasm of the host. It is therefore easy to distinguish the two, yet they blend into each other in such a way that it is impossible to determine sharply where the one begins and the other leaves off. Thus, the plasmodium in the host cell does not appear to be in a vacuole or to be separated from the host protoplasm by a membrane of any kind. The one seems to be somewhat miscible in the other, which is what might be expected if the surface tensions of the two plasms are equal. Czapek (3) has measured the surface tension of the protoplasm of widely separated groups and has found that in all cases it is approximately the same. This is an interesting point and deserves more detailed study.

The shape and general macroscopic appearance of the plasmodium as it attacks young potato tissue have already been described. Something should be said of its appearance in stained sections. The cytoplasm of the plasmodium is finely granular and shows a special affinity for the orange stain. Embedded in it in considerable abundance are the globular bodies that have already been mentioned. They stain very deeply with the gentian violet stain and often appear almost black. Various sizes are to be seen, which have been shown in Plate XXXIX, figures 3, 4, 5, 7, 8, and 9. They are also abundant in the photomicrographs shown in Plates XL, XLI, and XLII. The writer has not been able to discover what these bodies are or to observe that they have any specific structure. They are always present and very conspicuous in the infecting plasmodium and are carried with it into the potato cells. The considerable variation in their size and the intensity with which they take the gentian stain support the view that they may be encysted amœbæ that have been engulfed and are carried along. Starch grains, pieces of broken-down cell walls, and other foreign bodies are present in the infecting plasmodium. Nuclei can also be seen, but these are rather difficult

to stain. A few nuclei are shown in the infecting plasmodium in Plate XXXIX, figure 4. A nuclear membrane, nucleolus, and, in some cases, chromatin strands are to be seen. The nuclei stain much more readily after they have been carried into the host cells. Nuclear divisions have not been observed in the plasmodium before it enters the host cells.

In the way which has already been described the invading plasmodium infects a small pocket of tissue just beneath the epidermis. This varies from 1 to 2 mm. in diameter and from one to six cells in thickness. Its size determines the size of the powdery-scab sorus that is to result. If this small island of infected cells could be removed, it would leave a bowl-shaped cavity covered over by the epidermis.

Shortly after the cells become infected, they begin to grow very rapidly. Giant cells 5 or 10 times as large as the normal ones are soon to be seen. Instead of growing equally in all directions, the infected cells elongate, most of their growth being radially outward. This results in lifting the epidermis and finally in breaking through it. Plates XLI and XLII show the raised epidermis, while Plate XLIII, figure 1, gives a good idea of the appearance of the sorus after the epidermis has been ruptured. The torn edges turn back on all sides and give the appearance so characteristic of powdery-scab lesions. The individual cells at first become much enlarged. Their nuclei divide, sometimes mitotically, but much more often, it is believed, by direct division. In those cases where mitotic division of the nucleus occurs, a cell plate is formed and the cell becomes divided in the usual way. When the nuclei divide directly, the giant cells become multinucleate. Ultimately the giant cells are all cut up into smaller cells. Usually not more than five or six cells are produced by a single infected cell. Some of the giant cells and also some of the vertical rows of small cells that have resulted from the division of the large ones can be seen in Plates XLI and XLII. Plate XLI shows a vertical section through the edge of a young sorus. The epidermis has been slightly raised through the upward growth of infected cells. Just beneath the epidermis can be seen the dark intercellular spaces which were previously occupied by the infecting plasmodium. These spaces are now filled with débris left behind when the plasmodium entered the potato cells. The larger globules within the cells are the deeply stained bodies which are so common in the plasmodium. None of these bodies can be seen in uninfected cells. The smaller dark bodies are in most cases the nuclei of the parasite, and in some of the cells they can be seen clustered around the host nuclei. At this stage the host nuclei are spherical, and each contains a red-stained nucleolus. In this section most of the giant cells have been cut up into smaller ones, but two of them are still present. It can be seen that most of their growth has been radially outward. In Plate XLII can be seen a somewhat later stage than was shown in Plate XLI. Each host nucleus is embedded in a plasmodium. The intercellular spaces beneath the epidermis, which

were previously occupied by the infecting plasmodium, are quite clearly shown in this illustration.

The cells produced by divisions of the giant cells are all infected. They are approximately the size of the normal potato cells and seem to grow rather slowly. So far as has been observed, these cells rarely divide. Half a dozen, or even more, infected cells may result from one cell originally infected. The writer has never seen any indication that a plasmodium can pass from a growing infected cell into a healthy one.

Within the host cell the plasmodium is closely applied to the host nucleus, as shown in Plate XXXIX, figure 6. The host nucleus seems to be as thoroughly embedded in the plasmodium as are its own nuclei. Whether or not the fungus cytoplasm is in direct contact with the membrane of the host nucleus is difficult to determine, but, so far as appearance goes, this seems to be the case. It is interesting to see that the intercellular plasmodia do not kill the host cells, but merely stimulate them to increased growth and division. This relation indicates that *S. subterranea* is a rather highly specialized parasite.

Sometimes the host nucleus becomes much lobed and distorted; it often contains several nucleoli. The chromatin strands become abnormal in appearance or may even entirely disappear. In some instances, however, the host nucleus remains intact even after spore formation. It can then be seen embedded in the spore ball. The relation between the plasmodium and the host nucleus is an interesting subject, but the detailed description of the appearance of the diseased host cells will be left for some future time.

The plasmodium within the host cell is irregular in shape, as shown in Plate XXXIX, figure 6. The nuclei are rather evenly distributed and stain very readily. Each contains a large nucleolus. The nuclear membrane stands out clearly, and chromatin strands can be seen. The writer is of the opinion that these nuclei do not divide during the early stages of infection, but this point needs further study. Nuclear divisions in the plasmodium seem most common just after the giant host cell has divided into smaller ones. So far as has been observed, all the nuclei in a given plasmodium divide simultaneously. Mitotic divisions are the only kind that have been observed. The equatorial plate stage is shown in Plate XXXIX, figure 7. This is the stage most commonly met with in the preparations, but later stages are also abundant. Spindle fibers are clearly seen, but no astral rays or centrosomes have been observed. The writer agrees with Osborn (14) that nuclear division immediately precedes spore formation, but whether or not there are two successive divisions he is not prepared to say. In Plate XXXIX, figure 8, is shown a small portion of a plasmodium very highly magnified. A dense region can be seen around each nucleus. Appearances like this probably explain how Osborn (14) and others have been led to believe that the cells were infected by uninucleate amœbæ. It has generally been supposed that in

Spongospora, as well as in the other genera of the Plasmodiophoraceae, plasmodium formation takes place only a short time before spores are produced.

## OBSERVATIONS ON THE SECONDARY INFECTION OF TISSUE AROUND THE OLD SORI

One of the most serious aspects of the powdery scab as it appears in the United States is the dry rot that often sets in around the sori during the fall and winter while the tubers are in storage. The dry rot is familiar to all who are well acquainted with the powdery scab in this country. Both Melhus (12) and Morse (13) have made mention of it in their bulletins on that disease.

The demonstration of the existence of a saprophytic plasmodial stage in the life history of *S. subterranea* as given below at once suggested the possibility that this might be responsible for the so-called dry rot around the old sori. Pustules showing the rot in various stages were fixed in Flemming's stronger solution and later sectioned and stained with the triple stain. Sections from this material plainly show plasmodia in the shrunken areas around the old sori. Here they can be seen feeding on the potato cells and killing them. Plate XXXIX, figure 9, shows a plasmodium pushing down between the walls of mature cells in tissue near an old sorus. It causes the walls to become slightly swollen in much the same way as was described for the infecting plasmodium in the tissue of the young tuber. The walls appear to be softened and more or less gelatinous. They are not swollen as much as in the case of the cells in young tubers. The plasmodium can be seen pushing into the cells through the softened cell walls. Sometimes the openings thus made are quite small, but often a large portion of the wall is broken down. Once through the wall, the plasmodium seems to distribute itself throughout the protoplasm of the host. The different stages of this process have not been carefully studied, and it is not known in this case again just how the plasmodium gets through the limiting membrane of the host cell. The cells are quickly killed, and the cytoplasm and nucleus disintegrate, leaving only the starch grains. The starch seems to be acted on very little by the plasmodium, which is rather surprising in view of its action on the cell walls. As soon as one cell is killed, the plasmodium passes on to the next, and in this way cell after cell is destroyed. In front of the plasmodium can be seen the healthy tissue, while behind it is left a disorganized mass of broken-down cell walls, starch grains, and other débris. It seems that the plasmodium has no harmful effect in advance of the cells actually attacked, as would be the case if some poisonous substance were secreted, but that the cells are killed by actually becoming engulfed in it. The line between healthy and diseased tissue is very sharp.

Although the dry rot usually extends in all directions from the old sorus, the plasmodium is generally to be found only on one side. Nevertheless, the broken-down cells throughout the dry-rot area show the path that it has taken, indicating that the plasmodium moves about as it feeds on the tissue around and beneath the old sorus. It is not common for the rot to extend very deep, but plasmodia may occasionally be found as much as 6 or 8 mm. beneath the surface of the tuber. They usually feed in the tissue immediately beneath the epidermis, and whether or not they may go deeper into the tuber after it has been planted is a question that needs investigation. When infected potatoes are used as seed, the mother tubers undoubtedly harbor the plasmodia during the growing season.

In sections through some of the sori that are just beginning to show the dry rot very young plasmodia can be seen. Many of the spore balls in the base of the old sorus show germination, each cell of a spore ball producing a single uninucleate amœba. This method of germination agrees with that observed when the spore balls are placed in culture media. Sometimes, however, the walls of an entire spore ball disintegrate, leaving the amœbæ in such close contact with each other that they seem to fuse and can not be distinguished as separate bodies. Thus, a single spore ball may give rise to a baby plasmodium, several of which coming together form much larger ones.

The plasmodia in these secondary infections have much the same appearance as those which infect the young tubers. In favorable sections nuclei and the characteristic deeply stained globular bodies can be seen. The nuclei are rather poorly fixed, but a nuclear membrane and nucleolus can be distinguished.

It is interesting to compare the effect of the plasmodium on the growing cells in the young tubers with its action on the mature cells in the tissue around the old sori. The growing cells are not killed, but are stimulated to increased growth and division; and it is this growth and proliferation of cells that produces the raised sorus. The mature cells, on the other hand, are quickly killed, the tissue is destroyed, and shrunken, discolored areas result. The sorus is somewhat definite as regards shape, size, and number of cells attacked. Although the dry-rot areas are usually not very large, there seems to be no limit to the number of cells that may be destroyed when secondary infection occurs. The writer is of the opinion that the dry rot may be considered a mild form of the canker stage of *S. subterranea*. The canker stage by which deep holes are eaten into the tuber is, in all probability, an especially virulent form of the plasmodial stage in secondary infections. It is frequently mentioned in the European literature and is considered a serious form of the disease.

Other types of dry rot following *S. subterranea* and associated with the presence of *Fusarium* spp., *Phoma* spp., and other wound parasites also occur.

## OBSERVATIONS ON SPORE GERMINATION

The germination of the spores in the base of the old sorus has already been described. The writer has also studied their behavior in culture media. One of the first problems undertaken in this study of *S. subterranea* was that of spore germination. The spore balls were placed in a number of different substances, including distilled water, tap water, various sugar solutions, and potato infusions. The conclusion was soon reached that the spores germinate quite readily in media, but preparations favorable for demonstrating germination are not so easy to obtain. The demonstration of germination is, in fact, very difficult. The walls of the spores are so opaque that it is usually impossible to determine in unstained material whether or not a given cell has germinated. The amœbæ are quite small and hyaline. In liquid media they soon crawl away from the spore ball, and may easily be confused with protozoa.

The demonstration was finally accomplished by germinating the spores on agar media in Petri dishes. When a rather dry agar is used as the culture medium, the amœbæ remain, for a time at least, clustered around the mother spore ball. This gives an opportunity to observe the colonies produced by individual spore balls. When such material is fixed in Flemming's weaker solution, embedded in paraffin, sectioned and stained with the triple stain, it furnishes excellent opportunity for the study of germination. In this way permanent slides have been made which clearly show various stages of germination. Portions of germinating spore balls are shown in Plate XXXIX, figures 1 and 2. Figure 1 shows a small portion of a germinating spore ball from which some of the amœbæ are being set free. In one of the amœbæ shown in this illustration the nucleus can be clearly seen. The ungerminated spores are uninucleate. Figure 2 shows a small colony of amœbæ that have been set free through a partial disintegration of the old spore ball. The small deeply stained bodies are probably nucleoli; the nuclei can not be distinguished in these amœbæ.

In some cases the spore walls of the entire spore ball disintegrate, setting free as many amœbæ as there were cells in the spore ball. Generally, however, the amœbæ escape through openings in the walls of the individual spores, and the spore ball is left almost intact. Numerous spore balls showing nothing but empty walls can be seen in certain preparations. So far as has been observed, each spore contains only one nucleus and produces on germination a single, uninucleate amœba. The amœbæ are quite small, but under favorable conditions they grow rapidly and divide. They move about by means of pseudopodia. Cilia have not been observed.

A very good method for obtaining abundant germination is as follows: Mature spore balls taken from ordinary sori are dusted over the surface of a nutrient agar, such as Lima bean or potato agar in Petri dishes, just

before it hardens. They float on the surface of the medium and are held firmly in place when it hardens. The intimate contact thus obtained seems to give better germination than when the spore balls are dusted over the surface of the agar after it has hardened. Either method, however, will give good results.

In water or on agar containing no organic food material, such as sugars or proteids, germination takes place only after a considerable period of time. Under such conditions the spores may remain inactive for a month or longer, and even then a rather small percentage of them germinate, while on nutrient agar abundant germination is usually obtained. The spore balls in a given culture show considerable variation in the time required for germination. On a favorable medium many of the spore balls germinate within a few days, but in no case have all of the spore balls germinated, even when left on the agar for as long as two months. Why it is that the spores in certain of the spore balls fail to respond is a question that remains to be solved.

As the amœbæ leave the mother spore ball they crawl out over the surface of the agar. If the medium is allowed to become somewhat dry, they round up, produce a thick, rough wall, and while thus encysted are probably able to withstand various unfavorable conditions. The thick wall suggests that they would be quite resistant to desiccation, temperature variations, and toxic substances. They are generally uninucleate, but occasionally a binucleate cyst may be found. Under favorable conditions the cysts germinate. Through some means a hole is made in the thick wall and the amœba crawls out, and again encysts as soon as conditions become unfavorable. It seems that this process can be repeated an indefinite number of times, the cysts or resting spores furnishing a means by which the fungus may live over in the soil from year to year.

## OBSERVATIONS ON PLASMODIA PRODUCED IN CULTURES OF GERMINATING SPORES

Mention has already been made of finding plasmodia in the cultures of germinating spore balls. The question that at once arises is whether these plasmodia are produced through the fusion of the amœbæ of *S. subterranea*. The only way in which this problem can be definitely and finally solved is through infection experiments. An effort has been made to infect young potato tubers growing in a greenhouse by placing one or more plasmodia on them, but this work has not yet yielded satisfactory results. The method followed was to remove the soil from young tubers without breaking them from the mother plant or otherwise injuring them. The tubers are then washed in water, and a small piece of agar covered with a plasmodium similar to that shown in Plate XLIII, figure 2, is placed on each. In this way the plasmodium is brought into direct contact with the skin of the tuber. The tubers are then covered with

soil and left for a week or more before they are again observed. In a number of cases in such cultures the plasmodium passed through the skin of the tuber and killed some of the cells beneath the epidermis, but no typical sori have been produced.

Although the infection experiments have not yet given satisfactory results, the writer is strongly of the opinion that the plasmodium which he has in culture belongs to *S. subterranea*. These plasmodia have been obtained more than 100 times from cultures of germinating spores. All of the plasmodia are alike in appearance, and many of them have been seen to engulf the amœbæ of *S. subterranea* as they crawl about over the agar. A considerable number of the plasmodia have been isolated and grown separately on Lima-bean agar. By making transfers about once a week they can be kept in an active growing condition. Some of them have been obtained in pure culture, but such cultures soon become abnormal and die. Some evidence has been gained that if either certain bacteria or fungi be added to the culture, the plasmodium flourishes. This is in agreement with observations made by Pinoy (15) on other slime molds.

Some of the plasmodia have been induced to crawl up on glass slides, where they can be fixed by dipping the slides in Flemming's weaker solution. When stained with the triple stain, numerous nuclei can be seen. Near the center of each nucleus is a rather conspicuous red-staining nucleolus. In size and staining reactions, as well as in their general appearance, these nuclei resemble very closely those in the plasmodia within the living potato cells.

If a culture is allowed to become dry, the plasmodium encysts, as is common among the Myxomycetes. In some instances, when a plasmodium is transferred to a fresh medium, the streaming motion stops. The plasmodium then breaks up, and certain portions of the mass crawl slowly away and soon produce fruiting bodies that closely resemble those of Polysphondylium. This mass as it crawls along builds a central stalk composed of irregular-shaped cells, much as has been described for members of the Dictyosteliaceae. The stalk may lie flat on the medium for a distance of several millimeters, but sooner or later it bends upward and serves as a sporophore. The stalk, which varies both in length and in breadth, is sometimes composed of a single layer of cells, but often is five or six cells in breadth. These sporophores frequently branch several times. The spores are born in much the same way as has been described by Brefeld (1) for the genus Dictyostelium. They are regularly cylindric and about twice as long as broad. On germinating they give rise to amœbæ, pseudoplasmodia, and, later, to fruiting bodies like those just described. No other kind of fruiting bodies have been observed. Although it was not to be expected that a fungus with a true plasmodial stage would give rise to a pseudoplasmodium, this, nevertheless, seems

to be the case. When portions of encysted plasmodia are transferred to fresh agar, the cysts germinate and give rise to a large number of amœbæ, which form pseudoplasmodia and fruiting bodies like those above described.

## SUMMARY

(1) So far as known, the type of infection here described has never before been observed. Infection of growing potato tubers by *Spongospora subterranea* is accomplished not by separate amœbæ, as has previously been supposed, but through the action of a plasmodium which invades the tissue and infects a large number of cells at each point where it enters. The conception of a plasmodium invading healthy tissue, pushing down between the cells, and finally infecting them, is, it would seem, new to pathology. *S. subterranea* actually lives within the protoplasm of its host. In this respect it differs from most fungi and bacteria and offers an especially favorable opportunity for the study of the relations of host and parasite.

This account of the life history of *S. subterranea* raises many interesting questions regarding other members of the Plasmodiophoraceae. The manner in which infection takes place is unclear in the life history of all the members of this group. Do the amœbæ of *Plasmodiophora brassicae* produce plasmodia outside of the living cabbage cells? Are the cabbage cells attached by uninucleate amœbæ as was supposed by Woronin (17), or do they become infected in a manner similar to that above described for potato cells attacked by *S. subterranea*? The distribution of the diseased tissue in the roots of the cabbage suggests the latter method of infection.

(2) The cells in each little island of infected tissue are stimulated to abnormal growth and division.

(3) While the tubers are in storage the spores germinate in the base of the old sori and produce amœbæ which come together to form plasmodia that cause secondary infections.

(4) These plasmodia feed on the tissue around the old sori and cause a so-called dry rot, which is probably a mild form of the canker stage of the disease.

(5) The spores of *S. subterranea* germinate in culture media and each produces a single uninucleate amœba.

(6) When conditions become unfavorable the amœbæ encyst and go into a resting stage.

(7) The amœbæ seem to produce saprophytic plasmodia on culture media.

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## PLATE XXXIX

*Spongospora subterranea*

All figures were drawn with the aid of a camera-lucida and with Zeiss 2 mm. and 8 mm. objectives and oculars No. 4, 6, 8, and 12.

Fig. 1.—A small portion of a spore ball, showing the manner in which the spores germinate. × 1,500.

Fig. 2.—A portion of a spore ball and a small colony of amœbæ that have been set free by the disintegration of the spore walls. × 1,200.

Fig. 3.—A semidiagrammatic drawing of a section through a very young sorus, showing the infecting plasmodium as it pushes down between the cells. A few of the cells are already infected. Many of those in contact with the plasmodium are beginning to enlarge. The deep-staining globular bodies are distributed throughout the plasmodium. × 100.

Fig. 4.—An infecting plasmodium. The cells are being crowded apart as the plasmodium pushes down between them. The cell walls are becoming gelatinous and somewhat swollen, but are still intact. Deep-staining globular bodies and a few nuclei can be seen. × 1,000.

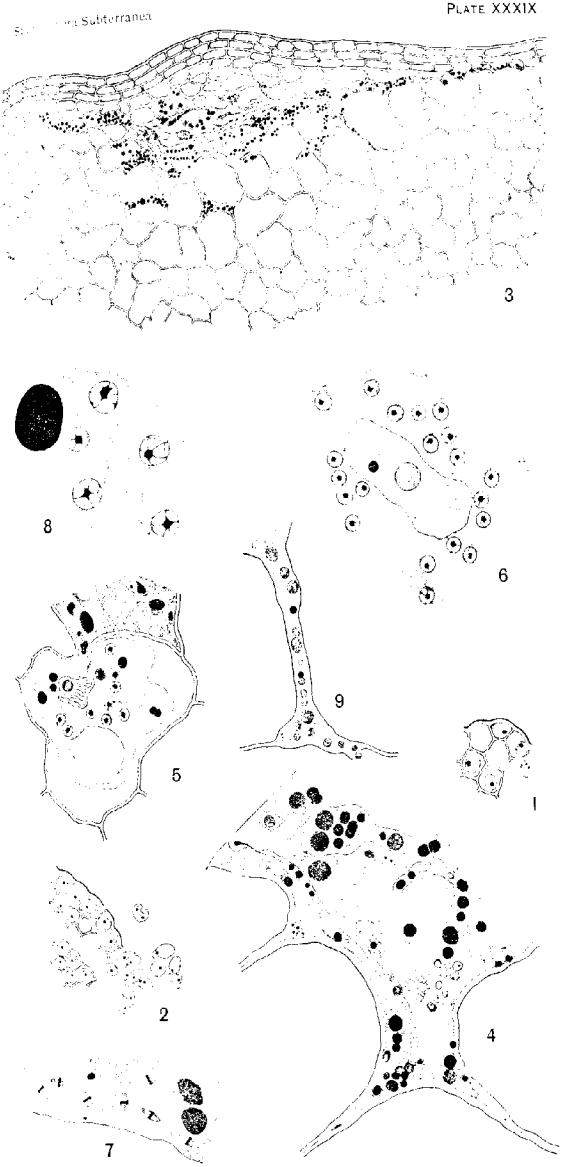
Fig. 5.—A potato cell becoming infected by a plasmodium of *S. subterranea*. The wall has been penetrated and the plasmodium is flowing into the cell. × 850.

Fig. 6.—A plasmodium closely applied to the host nucleus. The elongated host nucleus has a large and a small nucleolus, but is almost devoid of chromatin. × 1,200.

Fig. 7.—A small portion of a plasmodium. Simultaneous mitotic nuclear divisions are shown in metaphase. Spindle fibers are clearly seen, but astral rays and centrosomes are wanting. × 925.

Fig. 8.—Portion of a plasmodium within an infected cell, showing the dense cytoplasm around the nuclei and the clearer region between them. × 2,000.

Fig. 9.—A plasmodium pushing down between mature cells and causing secondary infection. The deep-staining globular bodies are present. × 1,000.


Subterranea
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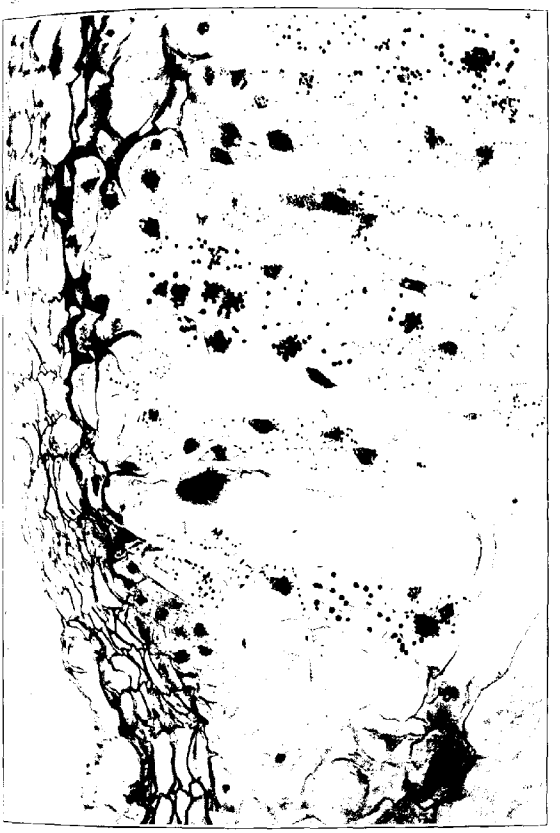
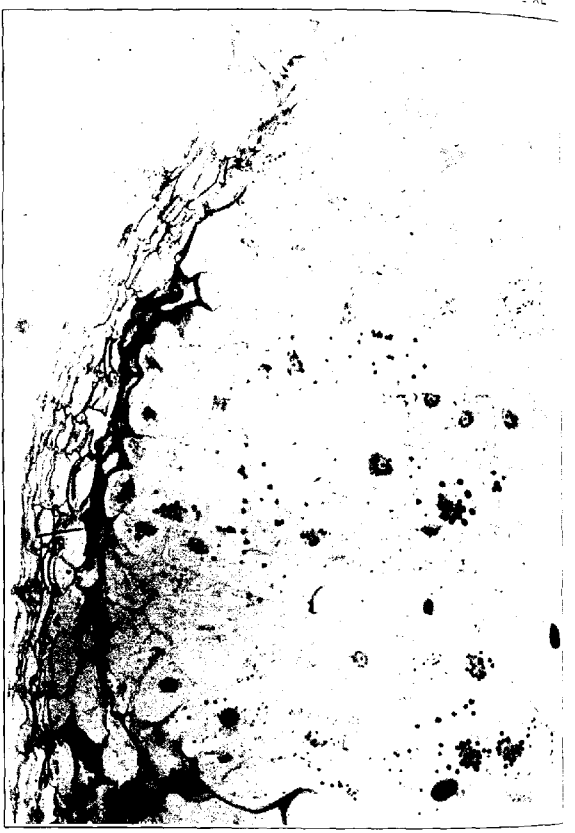
## PLATE XL

*Spongospora subterranea:* Vertical section through a very young sorus, showing the plasmodium as it pushes down between the cells. × 1,000.

## PLATE XLI

*Spongospora subterranea:* A vertical section through the edge of a young sorus, showing the intercellular spaces beneath the raised epidermis. These spaces were previously occupied by the infecting plasmodium. Two giant host cells are also shown in this illustration. × 150.





## PLATE XLII

*Spongospora subterranea:* Vertical section through the edge of a young sorus, showing the plasmodia in the potato cells and the intercellular spaces above which were previously occupied by the infecting plasmodium. × 150.

## PLATE XLIII

*Spongospora subterranea*

Fig. 1.—A vertical section through a sorus soon after it has broken through the epidermis. × 50.

Fig. 2.—A living plasmodium obtained from a culture of germinating spore balls. × 5.

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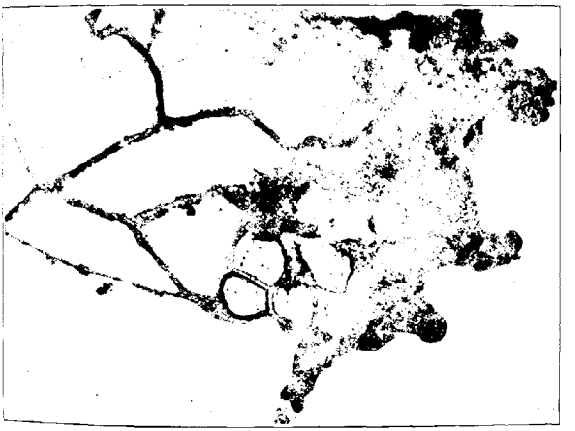

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